"EVALUATION OF HER2/NEU EXPRESSION IN CARCINOMA CERVIX"

DISSERTATION SUBMITTED TO

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

in partial fulfilment of

the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

TIRUNELVELI

APRIL-2015

CERTIFICATE

This is to certify that this Dissertation entitled "**EVALUATION OF HER2/NEU EXPRESSION IN CARCINOMA CERVIX**" is the bonafide original work of **Dr. N. SUPPULAKSHMI**, during the period of her Post graduate study from 2012 – 2015, under my guidance and supervision, in the Department of Pathology Tirunelveli Medical College & Hospital, Tirunelveli, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University will be held in April 2015.

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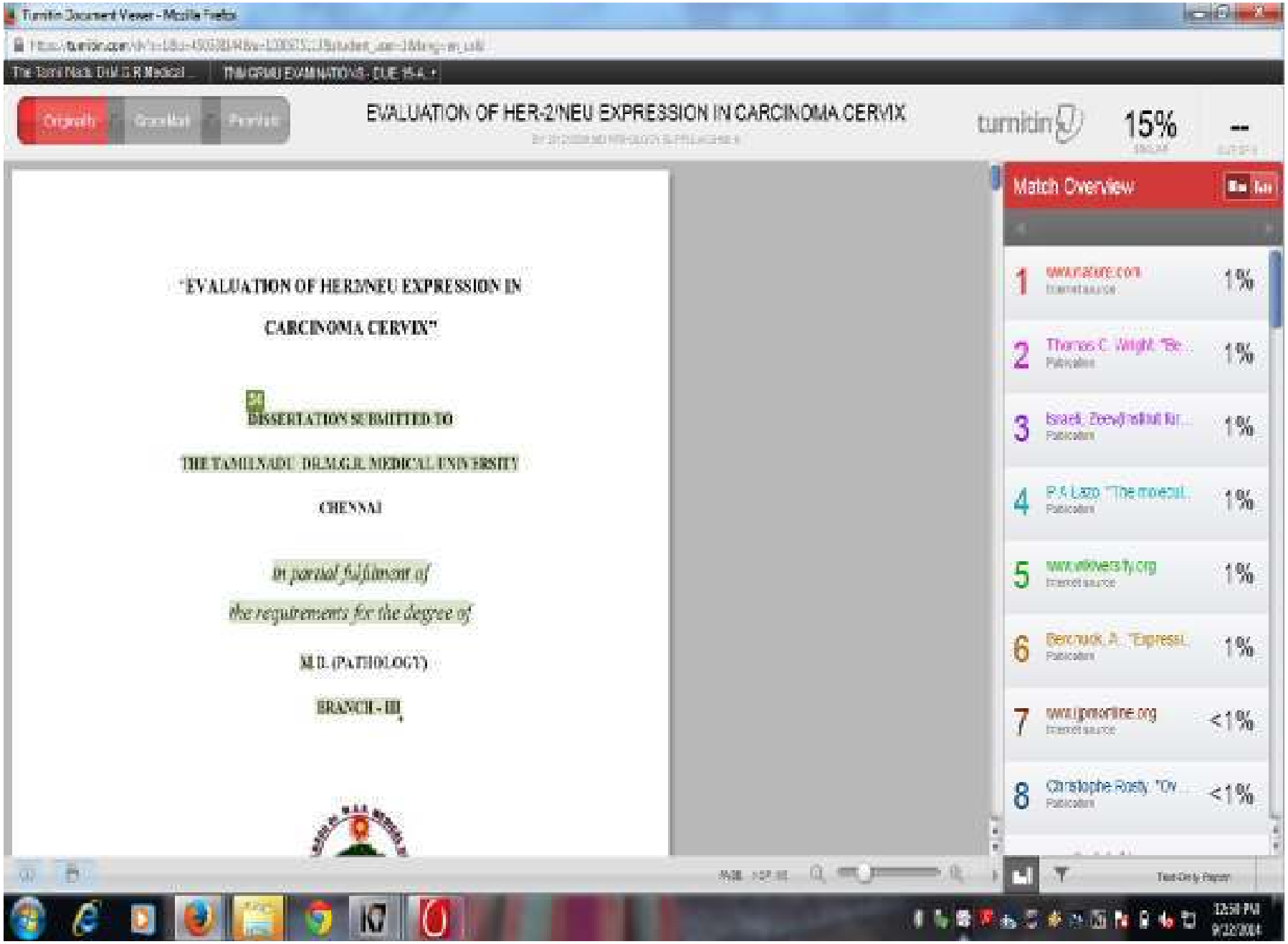
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"**EVALUATION OF HER2/NEU EXPRESSION IN CARCINOMA CERVIX**" submitted by me for the degree of M.D, is the record work carried out by me during the period of 2012-2015 under the guidance of **Prof. Dr. SITHY ATHIYA MUNAVARAH MD,** Professor of Pathology, Department of Pathology, Tirunelveli Medical College, Tirunelveli. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) Pathology examination to be held in April 2015.

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Dr. N.SUPPULAKSHMI

ABBREVATIONS:

1. HPV - Human Papilloma Virus
2. CIN - Cervical Intraepithelial Neoplasia
3. SCJ - Squamo-Columnar Junction
4. SIL - Squamous Intraepithelial Lesion
5. LSIL - Low grade Squamous Intraepithelial Neoplasia
6. HSIL - High grade Squamous Intraepithelial Neoplasia
7. LVSI - Lympho Vascular Space Invasion
8. MICA - MicroInvasive Carcinoma
9. LRIG 1 - Leucine Rich Repeats and ImmunoGlobulin like domains
10. FFPE - Fresh Frozen Paraffin Embedded tissue
11. IHC - ImmunoHistoChemisty
12. HIER - Heat Induced Epitope Retrieval
13. HER2 /neu - Human Epidermal growth Factor Receptor 2
14. DAB - Di Amino Benzidine
15. EGFR 2 - Epidermal Growth Factor Receptor-2
16. ASCO - American Soceity of Clinical Oncology
17. FIGO - International Federation of Obstetrics and Gynaecologists
18. COX 2 - Cycloxygenase-2
19. ICMR - Indian Council Of Medical Research

20. HLA - Human Leukocyte Antigen

21. CEA - Carcino Embryonic Antigen

22. TTF 1 - Thyroid Transcription Factor 1

23. PAX-2 - Paired boX gene 2

24. LOH - Loss Of Heterogeneity

25. TP 53 - Tumor Protein 53

26. MYCL 1 - V-myc Avian Myelocytomatosis Viral oncogene Homolog 1 lung carcinoma derived

27.CCND1 - Cyclin –D 1

28. GL 1 - Glabrous 1

29.HRAS -Harvey rat sarcoma viral oncogene

CONTENTS

ABSTRACT

Carcinoma of uterine cervix is the most prevalent cancer among Indian women. The HER-2/neu is the Human epidermal growth factor receptor-2 and it is a gene localized on chromosome 17q21 that encodes a growth factor receptor like molecule with tyrosine kinase activity and has a structure similar to that of epidermal growth factor receptor. Its expression has been detected in several human cancers and is believed to be associated with poor prognosis, aggressive biological behavior and metastatic potential. The present study is to evaluate the presence of HER-2/neu in carcinoma cervix, its pattern of expression and correlation with histological type, grade of tumor and clinical stage. IHC is an excellent technique to detect HER-2/neu expression in carcinoma cervix. Several studies on uterine lesions suggest HER2 positivity is directly related with higher grade and more aggressive tumor, and having poor prognosis. This study included 100 cases of carcinoma cervix and these were subjected to immunohistochemical staining for HER-2/neu oncoprotein. Out of 100 cases , only 17 cases were found to be positive for HER-2/oncoprotein. Among these 17 cases, 16 cases(94.1%) were of squamous cell carcinoma and 1 case was small cell carcinoma.It was also observed that the positivity rate varied with differentiation and staging, lymphnode metastasis and parametrial extension status. Poorly differentiated squamous cell carcinoma showed 25% positivity, whereas positivity

rate was 33.33% in cases of small cell carcinoma and was 50 % in stage III carcinoma. Also there was positivity rate of 33.33% in parametrial extension cases and 55% of positivity in lymph node metastatic cases. Thus HER-2/neu expression in Carcinoma cervix correlates with clinical stage, lymph node metastasis, and parametrial extension.

KEYWORDS: Carcinoma cervix, IHC, HER-2/neu

INTRODUCTION

Carcinoma of uterine cervix is the most prevalent cancer amongst Indian females [1]. In the developing countries, the most common cause of cancer mortality among female population is due to carcinoma cervix. Cervical carcinoma is the second most frequent malignancy in women worldwide. It is the major health problem, faced by the women in reproductive age group [2].The annual incidence of cervical carcinoma is about 1,32,000 and mortality caused by it is about 74,000[3].Among the total number of cases in the world, Indian females contribute to about one fourth of cases.

The carcinoma cervix has histopathologically, well characterized precursor lesions (CIN), which slowly progresses to the well differentiated tumor. This transformation of cervical epithelial cells from CIN to carcinoma takes 10 – 15 yrs. During this transformation period , many important markers of tumor progression, are expressed. Thus carcinoma cervix, because of its long period of transformation, provides a great opportunity to study about the expression of biomarkers of tumor progression. Because of the above advantages, present study was designed to evaluate the expression pattern of HER2 neu ,in different grades of cervical precancerous lesion and cancer lesions to understand the role of HER2 neu in cervical carcinogenesis. Many researches are going on to investigate the role of oncogenes in the development of cancers and the prognosis of various

cancers. The development of cancer depends on multiple factors and this process includes the sequential activation of oncogenes and other genetic derangements. [4]. The c-erbB-2 proto-oncogene is also called neu and HER-2/neu gene. It is a gene localized on Chromosome 17q21 that encodes a growth factor receptor-like molecule with tyrosine kinase activity. It has a structure similar to that of epidermal growth factor receptor.(5,6,7) Several tumors express HER2 neu and their expression has been associated with poor prognosis, aggressive behavior and its metastatic potential(8) . The present study was conducted to evaluate the presence of HER2/neu in the tumors of uterine cervix, its pattern of expression and correlation with histological type, grade of tumor and clinical stage, wherever possible.

AIM OF THE STUDY

1) To evaluate the expression of HER-2/neu oncogene in the Invasive carcinoma of uterine cervix

2) To determine the correlation of HER2/neu expression with clinical stage of carcinoma cervix

3) To determine the correlation of HER2/neu expression with histological types and grades of carcinoma cervix.

REVIEW OF LITERATURE

CARCINOMA CERVIX :

EPIDEMIOLOGY:

Cervical cancer is one of the most common cancers among women worldwide (WHO 2009). Majority of cervical cancer cases today occur in the developing world. According to National Cancer Registry of ICMR the incidence in India is 14.42/100000 population with mortality rate 2.83/100000 population (ICMR 2004). The highest incidence rates were observed in Latin America and the Caribbean, sub-Saharan Africa, and south and south-east Asia. Before the introduction of screening programmes, the incidence of cervical carcinoma was higher in Europe , North America and Japan over past several decades.

The incidence rate have declined in both white and African American women, for the past few decades. Since 2004 ,rates have decreased by 2.1 % per year in women younger than 50 years of age and by 3.1% per year in women aged 50yrs and above(American Cancer Society,Cancer Facts & Figures 2012).The demography based risk factors include age, marital status, socioeconomic status and ethnic and religious groupings.

Cervical cancer is the first human cancer to be found to have infectious agent as a causative factor(Thomson et al 2008.). Among the risk factors of cervical cancer, human papilloma virus infection especially ,the high risk type of HPV and persistence of HPV infection is the most

important risk factor. Harald Zur Hausen was awarded the noble prize in 2008, for the discovery of HPV as a cause of cervical cancer.

Human papilloma virus have nearly about 150 types. Among these 150 types, they are classified into high risk type, probable high risk, and low risk.

- Nearly 15 types are classified as high-risk types -16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82
- 3 types as probable high-risk- 26, 53, and 66
- 12 types as low-risk - 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 (Walboomers, J.M et al 1999).
- Types 16 and 18 cause about 70% of cervical cancer . Together with type 31, they are the prime risk factors for cervical cancer (Munoz N ,et al .,2003).HPV infections of the cervix is of transient in nature. This transient infection triggers immune system and produce immune response, mainly cellular immunity. Cellular immunity is responsible for the regression of HPV infections. In few cases the viral replication is prevented and may lead to latent infection or this will turn into active infection when the immune status of the patient is compromised. Certain factors like immunosuppression, high parity, smoking, long term use of oral contraceptive pills may accentuate the HPV infection to cancer (American Cancer Society , Cancer Facts & Figures).

Human papilloma virus usually infects immature squamous cells rather than mature cells. In cervix, the immature cells are seen in basal layer, where there is a break in the epithelium, and also seen in metaplastic squamous epithelium present at squamo-columnar junction. So HPV infects, most commonly the squamo-columnar junction where there is immature metaplastic cells. Even though it infects immature squamous cells, they replicate in the mature squamous cells. Due to their replication , there is a cytopathic effect in the mature cells and is called as 'koilocytic atypia'. The koilocytic atypia is characterized by perinuclear vacuolation, dense- and irregular-staining peripheral cytoplasm, and an enlarged nucleus with an undulating (raisin- or prune-like) nuclear membrane and a rope-like chromatin pattern (Lee KR et al 1997),that is seen in the HPV infected mature superficial cells. Normally the mature superficial cells do not proliferate. Human papilloma virus ,in order to replicate in this nonproliferating squamous cells,they have to reactivate the mitosis. This is done by interfering with the function of Rb and P53 . Viral E6 /E7 proteins are critical for the oncogenic effect of HPV. (Schiffman M et al 2007).

EMBRYOLOGY:

The paramesonephric duct or the mullerian duct, which are formed by the invaginations of coelomic epithelium is responsible for genesis of uterus with cervix and the upper portion of vagina.

ANATOMY:

The uterus is divided into the corpus, isthmus, and cervix. The inferior most portion of uterus is the cervix and it protrude into the upper part of vagina.It is a fibromuscular organ lined by mucus membrane. The length of cervix varies from 2.5–3 cm in the nulliparous adult women . In the anatomical position, it has a slight angulation downward and backward. The cervix is divided into three parts- Endocervix, Ectocervix, and Transformation zone.

1. The Endocervix is the portion of cervix which extends from the internal os to the ectocervix and it has the endocervical canal. The mucus secreting columnar epithelium that lines the endocervical canal is thrown into folds. The mucus secreting epithelium form folds that often projects into the underlying stroma forming complex glands or crypts
2. The Ectocervix is the portion of cervix which extends from the squamo - columnar junction to the vaginal fornices. This portion of cervix is lined by non keratinising stratified squamous epithelium and is hormone sensitive. The portion of cervix in the vagina is called as

exocervix. It is limited by the anterior and posterior vaginal fornices. It has a convex elliptical surface. The exocervix is divided into anterior and posterior lips. The anterior lip is shorter than posterior lip. The external os is present in the center of the exocervix.

3. The Squamocolumnar Junction (SCJ) denotes the region where the squamous epithelium of ectocervix and columnar epithelium of the endocervix meet. This SCJ varies in its position throughout life as a result of metaplastic changes that takes place in the columnar epithelium of the cervix. The SCJ is usually present at the external os, before the puberty. After child birth, the SCJ moves to the ectocervix. After the menopause the SCJ moves back to the endocervical canal.

Histology:

The ectocervix is lined by squamous epithelium and endocervix is lined by columnar epithelium. Stroma of the cervix comprised of fibrous, muscular, and elastic tissue. Among the stromal connective tissue, fibrous tissue constitutes the major portion. About 15% of the stroma is comprised of smooth muscle. Smooth muscle is present mainly in the endocervix and is absent in ectocervix. Smooth muscle constitutes about 50-60% of the connective tissue at the isthmus, where it is concentrically arranged to form a sphincter.

ECTOCERVIX:

Histology:

The ectocervix is lined by mature non keratinized squamous epithelium. This squamous epithelium in normal conditions , do not have rete ridges. Squamous epithelium of ectocervix has three distinct zones. They are as follows:

1. Basal/parabasal or germinal cell layer, which is responsible for continuous epithelial renewal
2. Midzone or stratum spinosum, the dominant portion of the epithelium
3. Superficial zone, containing the most mature cell population

GERMINAL LAYER:

The germinal layer consists of two cell types. They are basal cells and parabasal cells.

BASAL CELLS:

The basal cells are present just above the basal lamina. Basal cells are about 10 mm in diameter, having minimal cytoplasm and oval nuclei .Nucleus of basal cells are arranged perpendicularly to the basal lamina.

PARABASAL CELLS:

Parabasal cells, compared to basal cells, are larger. Parabasal cells are present above the basal layer and it is 1-2 cells thick. The important function of both basal and parabasal cells are the regeneration of the epithelium. Therefore the receptor for growth factor of the epidermis are present in the

basal and parabasal cells. The growth factor receptors are HER-2/neu, estrogen receptor and progesterone receptors[9,10]. As the cells differentiate into intermediate squamous cells, the growth factor receptors are reduced in number. Basal cells, also have the stem cell function. Parabasal cells are the cells that actively takes part in the replication. As the parabasal cells are undergoing active replication, there is increased mitosis in this layer. Not only the mitotic figures , but also other proliferative markers like Ki- 67 antigen, and other cyclins are found in parabasal cells.

MIDZONE:

Above the germinal layer, is the midzone. The midzone composed of cells that undergoes maturation . They differ from basal cells in having increased cytoplasm and the size of the nucleus remains unchanged till the superficial layer. These cells when exfoliated , are termed as intermediate cells. Intermediate cells does not undergo mitotic division. The cytoplasm of intermediate cells is clear and vacuolated. This vacuolation is due to the presence of glycogen, which is per iodic acid Schiff -positive, and diastase-labile.

SUPERFICIAL ZONE:

This zone is present in the superficial layer of the cervical epithelium. It is composed of superficial cells which are flattened and have abundant, pink,eosinophilic cytoplasm with nuclei being small and pyknotic. The stroma of ectocervix composed of fibrous connective tissue, and there is no

glands in this region. Stroma occasionally extends into the epithelium to produce stromal papillae.

ENDOCERVIX

Histology :

The endocervix is lined by single layer of tall columnar , mucin secreting epithelium. The endocervical stroma consists of endocervical glands, which are formed by complex infolding of the lining mucin secreting epithelium. The glands of the endocervix belong to compound, tubular racemose, type of glands. The lining epithelial cells are tall, columnar and are uniformly arranged. These cells have their nuclei being placed basally and have fine, granular cytoplasm filled with mucin secretions. These lining cells are called picket cells because of their appearance resembling a picket fence. There is another kind of ciliated cells, which are non secretory in nature, are present in the endocervix. As these cells are ciliated, they help in distribution and mobilization of the endocervical mucus.[11] The other types of cells that are seen in endocervical epithelium, that can be demonstrated by histochemical stains, are argyrophil, neuroendocrine and argentaffin cells.[12] Serotonin is present in the argentaffin positive cells.

TRANSFORMATION ZONE:

The area where the stratified squamous epithelium and glandular, mucin secreting columnar epithelium meet is known as squamo-columnar junction of the cervix

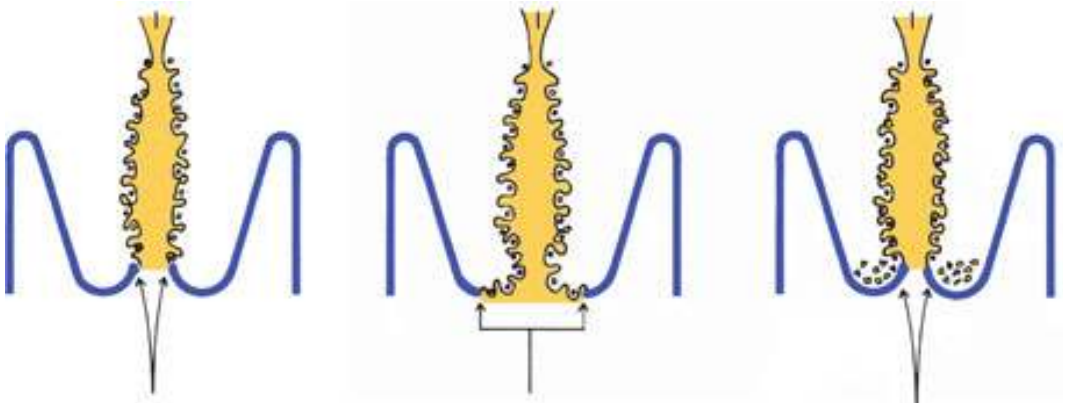

Fig : 1 **Fig : 2** **Fig : 3**

Fig : 1 - original squamocolumnar junction

Fig : 2 – endocervical erosion with original squamocolumnar junction

Fig : 3 – transformation zone with functional squamocolumnar junction

Actually, the adult cervix has two different squamocolumnar junctions. First one is the original squamo-columnar junction which is present since birth. Actually it is the place where native ectocervical stratified squamous epithelium meets the endocervical columnar epithelium. The second SCJ which is formed later in life ,either during menarche or during pregnancy. During this period, the cervix undergoes alterations in shape and size. Due to these changes, the columnar epithelium of the endocervix moves outward towards the ectocervix. This phenomenon

is referred to as eversion of endocervix. Normally the mucosa of the endocervix appears red and velvety, when compared to the mucosa of ectocervix which is pink and translucent. Due to the eversion of endocervix, during puberty or pregnancy, columnar epithelium in this area undergoes remodeling, and is replaced by metaplastic squamous epithelium .As this occurs, the histological squamocolumnar junction moves toward the external os. This newly formed squamocolumnar junction is called the physiologic, functional, or new squamocolumnar junction. The area that lies between the original squamocolumnar junction and the physiologic squamocolumnar junction is known as the transformation zone. The transformation zone comprises of metaplastic squamous epithelium.

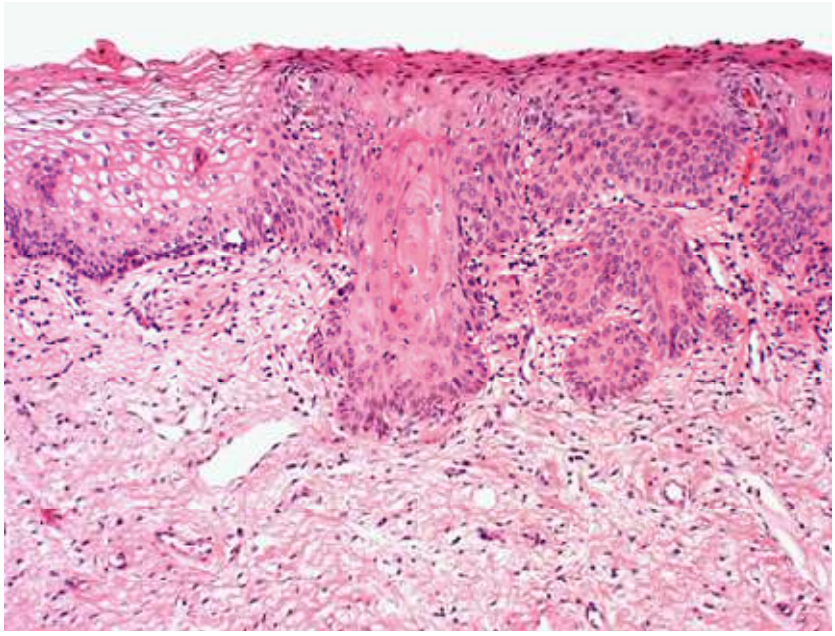

Fig :4 Histology of transformation zone

Nearly all the neoplastic lesions of the cervix arise from this newly formed physiologic squamo-columnar junction and cervical cancer precursors occurs within this transformation zone.

INVASIVE CARCINOMA

The invasive carcinoma of cervix is generally categorized into three main groups by World Health Organisation as follows:

1. Squamous cell carcinoma,
2. Adenocarcinoma, and
3. Other epithelial tumors.

Among all the tumors, the most common histologic type of tumor is Squamous cell carcinoma which accounts for about 70–80% of invasive carcinomas. The next common tumor to occur will be Adenocarcinoma and Adenosquamous carcinoma accounting 10–15% of all cases. Squamous cell carcinomas mostly arise from precancerous lesion and also arise from human papilloma viral infection, especially those which are persistent in nature [(13)]. Regarding precancerous lesion, nearly 10% to 20% of CIN III lesions if left untreated, eventually progress to frank malignancy. The transformation of CIN III to carcinoma may take many years and this varies from 3yrs to more than 20 years.[(14)]

The majority of squamous cell carcinomas occur in women who are between ages 40 and 55, with approximately 10% less than 30 years of age. Mean age for patients is approximately 51 yrs. At least 50% are stage I when

diagnosed. Of stage I tumors, 10% to 15% will be less than 5 mm in depth (stage IA). Survival is close to 100% for stage IA and diminishes with invasion beyond 5 mm and extension beyond the cervix (stage IIA) . The stage of cancer at the time of diagnosis is important as it is closely related to lymphnode metastases and thus determines the survival of the patient .Cervical carcinoma mainly spreads through superficial and deep lymphatics which finally drains into iliac, common iliac, obturator , hypogastric and sacral lymph nodes, as well as lymph nodes in the posterior bladder wall. The survival year of 5 yrs is reduced to 30% to 35% for stage III neoplasms.

RISK FACTORS:

The risk factors for cervical carcinoma includes viral causes and non viral causes.Viral cause includes HUMAN PAPILLOMA VIRUS infection.Other non viral causes are as follows:

- Multiple sexual partner
- Age
- High Parity
- Immune Status
- Certain HLA subtypes
- Use of oral contraceptives
- Use of nicotine
- Chlamydia infection
- Lesbainism

Human Papilloma viruses

HPV infection is the etiologic agent for the great majority of cervical epithelial neoplasms. Several studies including clinical and molecular studies have shown that HPV infection is strongly associated with cervical cancer and it directly influences the pathogenesis of cervical cancer. Over the past several years,it was observed that:

- HPV infection is ubiquitous in the young sexually active population,
- Frequency of infection peaks in the early reproductive years
- Usually the HPV infections are transient in nature ,which clears off and again it reappears, producing no cytological abnormalities.
- if the infection occurs persistently and it is produced by the HPV of same subtype, then there is high risk for the development of cervical cancer[15]

Cancer-associated ("high-risk") HPV types impose a broad gradient of risk, with HPV-16 conferring the greatest risk. Low-risk HPV types may confer risk as surrogate markers of "at-risk behavior." More than 100 human papilloma viruses have been discovered .Among the 100 types, nearly forty HPV types are found to infect genital tract. These HPV that are known to produce genital infections have been classified into low risk, high risk and intermediate risk types .The high risk has HPV has strong tendency to produce cervical carcinoma.[16] The HPV not only been associated with carcinoma but also produce preinvasive lesions.

TYPES OF HPV :

Kahn and associates followed women with mild cytologic atypia and correlated the outcome of CIN-3 at 10 years with HPV type. The relatively strong association between HPVs 16 and 18 and CIN-3 (15%to 18%) versus all other high-risk HPV types (3%) and low-risk HPV types (<1%) show HPV-16 as the prototypic high-risk type.[16]. A more recent study by Castle et al. showed that two high-risk or oncogenic HPV tests within approximately 1 year conferred a risk of CIN-2+ biopsy at 3 years of 17%, which increased to 41% for HPV-16[17] .

The relationships between HPVs and cervical neoplasia vary both for lesion grade and cell type.

- HPVs 6 and 11- are not associated with cervical carcinomas or high-grade squamous intraepithelial lesions. HPV-16: Nearly half of the HSIL cases have infection with HPV-16 and this infection is also strongly associated with squamous cell carcinoma of the cervix. HPV -16 is called the prototypic cancer causing virus
- HPV-56 – very few cases only (about 1 in 50) have this infection.
- HPV-18 – This type of HPV accounts for about 15% of squamous cell carcinoma of cervix.HPV 18 is also associated with adenocarcinoma and adenocarcinomain situ. HPV-18 is strongly associated with small cell neuroendocrine carcinoma.

A newly discovered HPV types –found to have uncertain or controversial association with cancer. They have been suggested to have presumably a low risk of cancer[18].

Prevalence rates of high-risk HPVs vary somewhat among different regions of the world and different countries. HPVs 16 and 18 are the dominant HPVs detected, ranging from 64% to 77% across different continents[19].The higher levels of viral DNA (viral load) correlate with risk of SIL[20] However, viral load will not discriminate low- from high-grade SIL because viral production is often higher in low-grade lesions. For this reason, viral load is not used as a predictor for HSIL.

Persistent infection by the same HPV type is strongly associated with risk of cervical neoplasia. Usually many types of HPV produce infection that are transient in nature. In various studies that dealt with several types of human papilloma virus, it had been found that persistent infections which were produced by same type of papilloma virus has highest risk of progression to cervical carcinoma[21]. In the studies done retrospectively on cervical cancer ,also shows high percentage of cases being infected with human papilloma virus.[22]

Elfgren et al. found that 92% of HPV-positive cases turned negative for infection over a 5-year period. But with HPV type 16 specifically, persistence of infection is highly related to the CIN and squamous cell carcinoma.

Conversely, Clavel et al. showed that approximately one third of high risk HPV positive women developed HSIL and two thirds of persistently high-risk HPV- positive women developed LSIL[23].Hopman et al. and Nobbenhuis et al.in their studies found that persistent infections with high risk HPV subtypes, had developed abnormalities in cervical smear, in more than 90% of cases .[24]

HPV intratypic variants exist and appear susceptible to the viral-like particle vaccines. Prior studies of different intratypic variants of HPV-16 showed that all were susceptible to the HPV-16 vaccine.[25]

Nonviral Factors:

Various host and behavioral factors are said to produce cervical cancer or HPV infection. They are as follows:

HLA type:

HLA that belongs to class II haplotypes (linked class II alleles) are generally related to cervical intraepithelial neoplasia and invasive carcinoma. Among this certain class II alleles are related to LSIL, HSIL, and full blown malignancy , but certain other type II alleles are protective in nature.[26] Still others have been associated with infection alone or certain HLA types are associated with cancers produced by certain HPV types, such as type 16. The studies observed that specific HLA class II haplotypes may influence HPV antigen presentation and the immune response to HPV infection, in turn

influencing the risk of developing invasive cervical carcinoma. The precise mechanisms underlying these associations is still under study.

***2.Age**:*

Young, sexually active women are at greatest risk for HPV infection and preinvasive cervical neoplasia. This risk gets reduced significantly with increasing age, which is associated with increasing risk of cancer . There is a reduced risk with the attainment of menopause. Thus the HPV infection risk is increased in young age and is reduced as the age increases. The progressive drop in risk with increasing age has been attributed to an effective immune response to the virus that follows the onset of sexual activity and exposure to HPVs. Protection is long lasting, given the low rates of HPV positivity in middle-age women.

***3.Immune status**:*

Immunodeficient status in certain conditions like HIV infection, post transplantation, immunosuppresed individuals have increased risk of developing cervical carcinoma. In post transplant therapy, there is deficient cell mediated immunity and therefore increased risk of cervical cancer. HIV-infected individuals are prone to HPV infection, persistent HPV infection, and a higher risk of precursor lesions. The prognosis of cervical cancer in HIV infection appears to be worse in patients with severe immunodeficiency. The risk of cervical neoplasia or HPV infection in immunosuppressed individuals is well established. The patients with

transplants have higher risk of HPV positivity.[27] Cancer rates are higher in this population, and in general anogenital cancers occur at a younger age. The rate of the virus infection in the immunosuppressed patients were found to be increased 9 folds when compared to the general population and much more higher nearly about seventeen times, in matched immunocompetent patients[28] .About one third of HPV infected individuals and nearly 50% of cancer patients have involvement of multiple sites in the lower genital tract.

4.The male partners*:*

The role of the male partner can be summarized as follows: It has been proposed that if the male partners have multiple sexual partners, it influences the risk of cancer development, even though it is of lesser degree. There is little evidence that the male partner re-infects the woman, at least with the same HPV type. Krebs and Helmkamp showed that treating the male partner did not influence risk of recurrent genital warts.[29] The implication of this study is that partners are not reinfected by the same virus, consistent with a functioning immune system. Kjaer et al. found that repeated sexual contacts of the male partner with other women did not increase risk independently, whereas a history of genital warts and absence of condom use did increase the risk. Condoms protect against HPV infections and significantly reduce the risk of cervical HPV infection. Circumcision reduces the risk of infection in the male partner and his female contacts, producing a relative risk of 0.65

in the male. Other infections, such as syphilis and HIV, are also significantly reduced in circumcised men[30].

5.Oral contraceptives.

The use of oral contraceptives has shown to have increased risk for the development of carcinoma cervix.There is a strong theoretic basis for hormones influencing epithelial growth and susceptibility to neoplasia in the cervical ransformation zone[31]. Oral contraceptive use and plasma levels of hormone have been associated with cervical neoplasia[32]; however, Kjellberg et al. and Coker et al. noted an association between OCPs and cervical neoplasia but the association disappeared when HPV was taken into account[33]. There is a strong association between hormonal replacement and cervical adenocarcinoma. A recent large meta-analysis postulated a relative risk of 1.90 with use of oral contraceptive pills greater than 5 years and a decline in risk with cessation of use.[34]

6.Smoking.

Smoking increases the risk of cervical neoplasia, but the mechanism is unclear. Kjellberg et al. noted a strong (independent of HPV) association between smoking and cervical neoplasia[35] .The theoretic basis is presumably the presence of DNA adducts in the cervical mucus, exposing the transformation zone mucosa to carcinogens. Genetic polymorphisms that theoretically do not reduce adduct formation have also been implicated. Lacey et al. showed a positive relationship to squamous but not

adenocarcinoma.[36] Deacon et al. also showed a dose- response relationship[37] A retrospective study in a Nordic population showed an odds ratio of 2.7 for women with nicotine in their serum after controlling for the presence of HPV-16/18 antibodies.[38].

7.*Chlamydia infection*.

The association between chlamydia infection and cervical neoplasia is controversial. Anttila et al. found that evidence of infection with chlamydia serotype G conferred a significant risk of cervical cancer[39]. Several studies have shown a relationship between genital infections and HPV or HSIL

8.*Women who have sex with women*.

Risk of cervical neoplasia is increased in women who are lesbians. Studies have shown high rates of sexually transmitted infections in women who have sex predominantly with other women,but the occurrence of genital condylomata were low. However, the risk of developing HSIL was not determined. Two reports have shown that CIN-2 may occur in lesbian[40] In summary, a multitude of factors, viral and host related factors, influences risk of cervical neoplasia before, during, and following exposure and lesion progression. .

TABLE no:1 Risk factors for HPV infection and cervical neoplasia: (35,26,33,28)

FACTORS	OUTCOME VARIABLE	POSITIVE/ NEGATIVE
Young age at coitus	HPV + HSIL	Positive
Sex partner>2	HPV INFECTION	Positive
Recent sexual activity	HPV INFECTION	Positive
Male promiscuity	CARCINOMA	Positive
Parity (>7)	CARCINOMA	Positive
Increasing age	HPV	Negative
Cervicitis	HSIL	Positive
Genital infections	HPV	Positive
Chlamydia infections (type G)	SQUAMOUS CELL CARCINOMA	Positive
Barrier contraception	HSIL/ CARCINOMA	Negative
Smoking	CIN-2/3 or CIN-3/carcinoma	Positive
Oral contrceptives or unopposed estrogens	ADENOCARCINOMA	Positive
Oral contraceptives	CARCINOMA	Positive
High risk HPV	CARCINOMA	Positive
Persistent infection with high risk HPVS	HSIL, CARCINOMA	Positive
High viral load(HPV)	HSIL	Positive
HLA -B 07 & HLA- DQB1-302	HSIL/ CARCINOMA	Positive
Transplantation	CERVICAL	Positive

	CARCINOMA	
HIV INFECTIONS	Index of HPV	Positive (2 fold)
	Persistent HPV	Positive (6 fold)
	SIL	Positive (4 fold)
	Persistent of SIL	Positive (4 fold)
	HSIL/ CARCINOMA	Variable (0-15 fold)

HISTOPATHOLOGY:

SQUAMOUS CELL CARCINOMA:

Invasive squamous cell carcinoma have variying gross presentations.They may present either as focally indurated lesion, or ulceration or as an elevated lesion, that easily bleeds. Most of the early carcinomas of cervix are present within the transformation zone.The advanced carcinomas of cervix mostly present either as a exophytic mass or an endophytic growth. The exophytic growth usually have a papillary or polypoid mass. Endophytic growth of carcinoma cervix grossly appears as ulceration or as nodules. Generally these growth occurs in the endocervical canal and often they invade into the underlying cervical stroma.

Microscopy:

On histopathological examination, squamous cell carcinoma is characterized by invasion of neoplastic squamous cells into the stroma , forming anastomosing cords in the stroma. The margins of the infiltrating

cords of malignant squamous cells, are irregular and have ragged edges.The invasion of the tumor into the stroma occurs either as an individual tumor cells or as a large mass that totally replaces the stroma.

The centre of the infiltrating malignant squamous cells undergoes necrosis or sometimes they undergo intense keratinisation. The individual tumor cells are large, polygonal in shape or sometimes oval with acidophilic cytoplasm. Usually the cell membranes of the malignant squamous cells are prominent and the intracellular bridges may or may not be present. The tumor cells exhibit relatively uniform nuclei, with occasionally showing pleomorphism. The nuclear chromatin being coarse and clumped. Generally the mitotic figures are seen, and often abnormal forms are also seen.

HISTOLOGICAL TYPES:

The squamous cell carcinomas of the cervix were classified depending upon the type of cell that is predominantly seen. The recent classification by WHO(Annexure -2) of cancer cervix classifies small cell carcinoma with neuroendocrine features as a separate entity. The invasive squamous cell carcinomas is broadly grouped under two major groups,namely keratinizing and nonkeratinizing[41].

KERATINISING SQUAMOUS CELL CARCINOMA:

The squamous cell carcinoma of keratinizing type is a lesion composed of malignant squamous cells arranged in cords or nests that show variation in shape and size . The characteristic feature of this type of

carcinomas is the keratinisation of the tumor cells. The evidence of keratinisation is appreciated by the presence of keratin pearls within the epithelium, or the presence of kerato hyaline granules or the individual keratinized cells and nests of squamous cells with central keratinisation. It has been proposed that even one keratin pearl, if present , is enough to categorize this tumor as a keratinizing carcinoma. The nests of squamous cells are arranged in a concentric fashion and they undergo keratinization to form the characteristic keratin pearls. The malignant squamous cells that doesnot form pearls, often show abundant acidophilic cytoplasm with their intercellular bridges being prominent.These changes are referred to as individual cell keratinisation. The tumor cells have enlarged nuclei and the mitosis is not high. These tumors are usually termed as well differentiated carcinoma and they have a pushing border of invasion.

NON KERATINISING SQUAMOUS CELL CARCINOMA:

As the name implies, these tumor does not have characteristic keratin pearls. This tumor histologically shows the nests of malignant squamous cells that undergoes individual cell keratinisation. Individual tumor cells are large with indistinct cell margins and exhibit greater degree of nuclear pleomorphism , with round to oval nuclei,having coarse,clumped chromatin. They often have numerous mitotic figures.

These tumors have an infiltrative border and often associated with inflammation. These tumors are referred to as moderately differentiated .

MICROSCOPIC GRADING:

Recently squamous cell carcinomas are graded histologically as follows:

1. Grade I-Well differentiated
2. Grade II -Moderately differentiated
3. Grade III - Poorly differentiated

In general, the most commonly occurring tumor belongs to grade II, that is moderately differentiated squamous cell carcinoma. Next frequent tumor would be grade III(poorly differentiated), followed by grade I (well differentiated)

Well-differentiated squamous cell carcinoma:

This carcinoma is characterized by the presence of keratin pearls, individual cell keratinisation and prominent intercellular bridges.

The tightly packed cells have irregular , hyperchromatic nuclei. Mitosis is seen at the advancing margin of the tumor.There is often inflammatory reaction in the stroma , with occasional giant cells of foreign body type.

Moderately differentiated squamous cell carcinoma:

The grade II tumor cells are large, with irregular nuclei showing moderate pleomorphism with decreased amount of cytoplasm. They have indistinct cell margin and intercellular bridge. Keratin pearls are absent. Tumor cells show individual cell keratinization and the increased mitosis.

Poorly differentiated squamous cell carcinomas :

The tumor cells show severe pleomorphism with scanty cytoplasm and oval, hyperchromatic nuclei. Occasionally there are giant cells with bizarre nuclei can be seen. There is complete absence of keratinisation. Rarely the cells may appear spindle shaped. They have increased mitotic rate and areas of necrosis

VARIANTS OF SQUAMOUS CELL CARCINOMA:

Verrucous carcinoma

It is a rare variant , which is a highly differentiated type of squamous cell carcinoma .On gross it appears to be polypoid in nature, and have an extremely well-differentiated cytologic appearance, and a capacity for local invasion but not for metastatic spread. Some cases have been found to extend into the endometrial cavity.[42]It should be distinguished from condyloma acuminatum and squamous cell carcinoma with a prominent papillary pattern of growth .

Spindle cell carcinoma

(sarcomatoid carcinoma; squamous cell carcinoma with sarcoma-like stroma; carcinosarcoma)

This carcinoma is morphologically analogous to the homonymous tumor in the upper aerodigestive tract.[43] It may contain osteoclast like giant cells,[411] and there is often evidence of HPV infection.(44) The epithelial component of this tumor, when present, this is usually of squamous type.

Basaloid (squamous cell) carcinoma

Basaloid carcinoma is characterized by prominent peripheral palisading, an infiltrative growth pattern, and minimal stromal reaction[45].

This tumor is aggressive and the differential diagnosis for this are the adenoid cystic carcinomas and adenoid basal carcinomas.

Lymphoepithelioma-like carcinoma

This carcinoma resembles its counterpart in the upper respiratory tract by virtue of the large size of the tumor cells, vesicular nuclei with prominent nucleoli, syncytial appearance, and heavy lymphocytic infiltration.[46]. Some cases have been found to secrete beta HCG

Transitional cell (urothelial) carcinoma:

This lesion having an appearance similar to the tumor located in the bladder or ovary . It needs to be distinguished from inverted transistional cell papilloma and papillary squamous cell carcinoma.

MICRO INVASIVE SQUAMOUS CELL CARCINOMA:

This is also called as early invasive carcinoma of cervix. This tumor lies in between the spectrum of CIN and well established invasive carcinoma. According to FIGO staging, it is placed under stage IA. This is defined as tumor less than or equal to 3mm in depth and 7mmin length. There is no capillary or lymphatic invasion .Diagnosis of MICA can be made only by histopathological examination.

The criteria for diagnosis are desmoplastic reaction of the stroma, well matured squamous cells, and loss of polarity, necrosis in the lumen. Common age of presentation, falls between 35 – 46 yrs. It constitutes about 20% of all cancer of cervix. High grade SIL with endocervical crypt involvement is associated with MICA.

CERVICAL INTRAEPITHELIAL NEOPLASIA:

The term cervical intraepithelial neoplasia includes all squamous cell alterations that is produced in and around the transformation zone and usually associated with human papilloma virus infection. This is considered to be the precancerous lesion of the cervix. They have been classified as mild , moderate and severe dysplasia according to oldest classification. According to WHO ,it has been classified as CIN I, CIN II, CIN III. Bethesda system classifies the lesions based on the cytology as LSIL, HSIL. The characteristic feature of CIN was that the cellular atypia did not involve the entire epithelium and might involve one third or two third of the cervical epithetlium. Other distinguishing feature was that it did not have invasion of stroma neither there was disruption of basement membrane.

Actually mild dysplasia corresponds to CIN I, moderate dysplasia corresponds to CIN II, and CINIII includes both severe dysplasia and carcinoma in situ.

TABLE no : 2 Terminologies for cervical cancer precursors:

Older classification	WHO classification	Bethesda system terminology
Mild dysplasia	CIN I	LSIL
Moderate dysplasia	CIN II	HSIL
Severe dysplasia/carcinoma in situ	CIN III	HSIL

CIN- I:

In this category, nuclear atypia which is usually mild and nuclear abnormalities are present in the lower one third of cervical epithelium. Mitosis may not be prominent. Nuclear atypia with perinuclear clearing is called koilocytic atypia. The cells in the upper two thirds of the epithelium, are usually matured.

CIN – II:

Here nuclear abnormalities and nuclear atypia is seen in lower two third of the cervical epithelium. Mitotic figures are limited to the basal two third layers of cervical epithelium and occasionally have abnormal mitotic figures.

CIN – III:

In this type , there is marked nuclear abnormalities and atypia is seen through entire thickness of the cervical epithelium with absence of

maturation throughout. As it is high grade, it has increased number of mitotic figures, as well as abnormal forms.

Human papilloma viruses are strongly related to cervical intraepithelial neoplasia, especially with high risk strain of HPV. HPV infection interfere with and alters the cell cycle. The cell cycle biomarkers are helpful in distinguishing CIN from other atypia. Ki- 67 is a cell proliferation marker,which is normally seen in the suprabasal cells. Another recently identified marker of CIN is P 16 , a cyclin dependent kinase inhibitor.

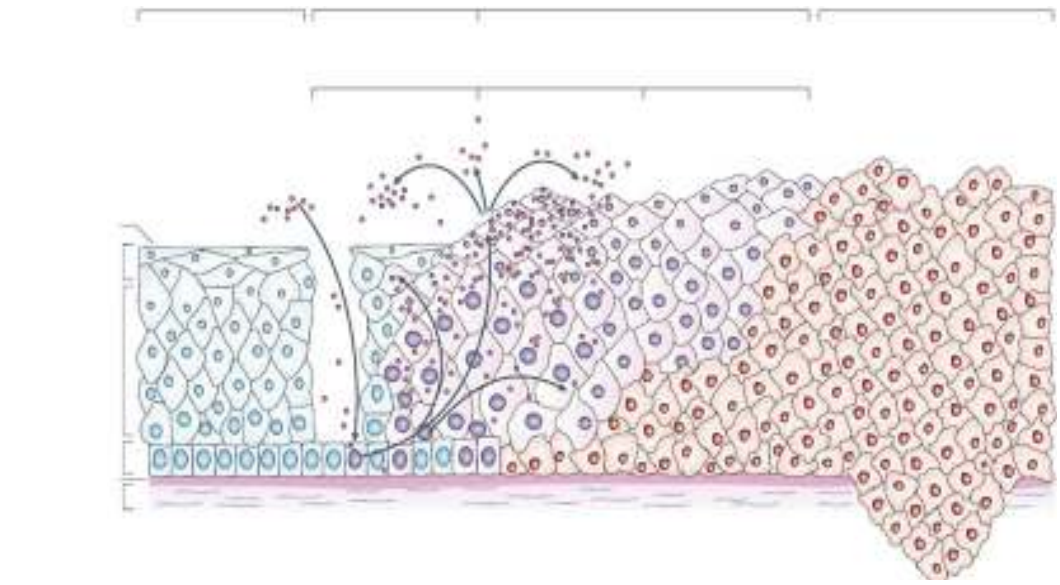

Fig : 5 Evolution of CIN(SIL) and invasive carcinoma from normal cervix epithelium

As CIN is closely related to HPV infections, HPV linked cytologic abnormalities like koilocytic atypia, multinucleated cells, and dyskeratosis are seen. The cytologic changes like enlargement of cells, with increased

size of the nucleus, often nucleus showing binucleation associated with irregular nuclear membrane and hyperchromasia.

Hyperplasia of reserve cell with atypia squamous metaplasia with atypia are regarded as CIN variants.

Cytopathology:

CIN is graded cytologically depending on the nucleus morphology.

CIN I : Epithelial cells show little enlargement of nucleus, which occupies about one third of the cell, mild degree of anisokaryosis and hyperchromasia. Nuclear chromatin is distributed evenly and have fine granules.

CIN II : There is marked variation in shape and size of the cell, as well as the nucleus. The nucleus occupies about half of the cell, and the chromatin is irregular and have moderate hyperchromasia.

CIN III: The nucleus occupies more than two third of the cell with hyperchromatic nucleus. The chromatin is irregular and have coarse granules.

Carcinoma in situ : The tumor cells are arranged in syncytial aggregates or as a single cells , characterized by nuclear overlapping and cell margins are indistinct, with scant cytoplasm , oval to round nucleus.

ADENOCARCINOMA:

Primary adenocarcinomas make up 5–15% of all carcinomas of the cervix. Incidence is on the rise in the general population, particularly in

young women.[47] An association has been found between the long-term use of oral contraceptives and the development of endocervical neoplasia in young patients,[48] The tumor shows no distinguishing gross features.

Microscopically, the most common pattern is that of a well-differentiated glandular pattern with mucin secretion, some of which can leak into the stroma[49] However, the degree of differentiation varies, and poorly differentiated forms exist. In addition to the mucin-secreting appearance, which recapitulates the histology of the normal endocervix, cervical carcinomas can have an endometrioid or a serous (papillary) appearance.

Endometrioid adenocarcinoma

It looks similar to its counterpart in endometrium. Endocervical type of endometroid adenocarcinoma is usually associated with HPV infection and adenocarcinoma in situ. This cancer occurs in younger age group patients. The factors that favours the endocervical origin are: the majority of the tumor occurs in the endocervical region, presence of cervical mass, its association with HPV infection, and adenocarcinoma in situ, younger age.

Serous (papillary) carcinoma:

The Primary serous adenocarcinoma of the cervix is extremely uncommon. Usually it has bimodal pattern of presentation that is below 45 yrs and above 65 yrs. Grossly it appears as exophytic or polypoid mass.

These tumors are histologically similar to papillary serous adenocarcinoma of any FGT tumor.

They have complex branching papillae, with cellular tufting , micropapillae and slit like spaces are seen. Numerous mitotic figures are seen and are commonly associated with villoglandular adenocarcinoma.

Adenoma malignum

Synonym: minimal deviation adenocarcinoma

This tumor type is a rare entity, which is well differentiated mucinous tumor. Minimal deviation adenocarcinoma is usually associated with mucinous ovarian carcinoma and sometimes with Peutz- jeghers syndrome.[50]

Histologically, they composed of infiltrating , irregular glands, and sometimes extend to the outer third of cervix[51]. The neoplastic glands are lined by mucinous epithelial cells with pale eosinophilic cytoplasm and small nucleus located basally with few cells showing prominent nucleoli , with focal desmoplastic reaction of the stroma.The neoplastic glands secrete neutral mucin. They are rarely associated with HPV infections.

Villoglandular Adenocarcinoma:

This is an uncommon variant of adenocarcinoma. Tumors often have an exophytic polypoid or papillary growth. The characteristic feature of this tumor is the surface papillary component which has finger-like papillae in the background of fibrous spindled stroma. The papillae is lined by stratified

columnar epithelium with nucleus showing mild nuclear atypia. This type of carcinoma is usually associated with HPV infection of high-risk strains namely types 16 and 18 and also is accompanied by adjacent adenocarcinoma in situ.

Adenosquamous (mixed) carcinoma

The mixed carcinoma has elements of both squamous and glandular components. This carcinoma arise from sub columnar reserve cell[52]. It is related to pregnancy and it resembles mucoepidermoid carcinoma of salivary gland and is said to have poor prognosis.

Glassy cell carcinoma:

It is a special type of adenosquamous carcinoma as this cancer exhibits feature of both squamous and glandular components. This tumor occur in a younger age group than cervical adenocarcinoma. They present as a large, bulky, fungating mass grossly . Histologically the tumor consists of tumor cells arranged in diffuse sheets and nests of large cells, with abundant eosinophilic finely granular cytoplasm giving a ground-glass appearance,with enlarged nucleus and prominent nucleoli with prominent cell margin. The stroma is infiltrated by chronic inflammatory cell infiltrate, which includes eosinophils and plasma cells. There are numerous mitosis seen. Generally, it has worse outcome.[53]

Mucinous carcinoma:

Mucinous carcinoma of the endocervix remains an ill-defined concept. Its immunophenotype can be of gastric or intestinal type, including immunoreactivity for CK7, p16, and a new marker known as HIK1083.[54] This tumor type is said to run a more aggressive clinical course than the usual type of endocervical adenocarcinoma.[55]

Adenoid cystic carcinoma:

This variant is rare in cervix, commonly occurs in postmenopausal age group patients(56). Grossly it is friable polypoid or ulcerated mass . It has worse prognosis as it has high rate of recurrence focally and also distant metastasis.

Histologically, cervical adenoid cystic carcinoma has similar features as that of its counterpart in salivary gland tumor . Histologic hallmark of this tumor is its cribriform pattern with an empty space surrounded by pallisading nuclei. The empty space sometimes show eosinophilic secretions that is hyaline or mucinous in nature. This tumor has numerous mitotic figures, and extensive necrosis. Individual tumor cells show pleomorphism and the stroma exihibit dense desmoplastic reaction. Immunohistochemically they are positive for S-100 and HHF35.

Adenoid basal carcinoma (Adenoid basal epithelioma) tumor; epithelioma) :

It is a low grade lesion. It has similarities with adenoid cystic carcinoma. The prognosis is excellent and generally do not have metastasis or recurrences. They doesnot produce mass lesion[57]. In some instances, they are associated with adenosquamous carcinoma, adenoid cystic carcinoma or cacinosarcoma. They are related to HPV infection of high risk strain and with HSIL. They present histologically as discrete rounded nests and islands of atypical squamous cells with prominent pallisading of nucleus or as nests of basaloid cells or basaloid cells admixed with acinar or glandular structures.

Clear cell carcinoma

Synonym: formerly called as Mesonephric carcinoma

This tumor has bimodal age distribution. They may occur sporadically or due to the use of diethylstilbesterol in utero. Microscopically, the tumor composed of neoplastic glands that are lined by large cells with abundant clear cytoplasm, and hyperchromatic nuclei. They also may be arranged in tubulo-cystic pattern , or papillary pattern or in solid pattern. Hobnail cells can be frequently made out. This cancer arises from mullerian ducts. This variant has good prognosis.

Mesonephric (adeno)carcinoma

These carcinoma arise from mesonephric remnants. These tumors grossly present as exophytic mass or polypoid mass or the enlargement of cervix diffusely, which is referred to as the barrel cervix. This cancer involves the entire thickness of the cervical epithelium and has an infiltrating margin, and occasionally it may erode the cervical mucosa. Mesonephric hyperplasia is usually present. They present with various different pattern like ductal, tubular pattern.

Other patterns are reteform , solid and sex cord pattern, rarely show spindled[58] pattern also. The spindled pattern , histologically looks similar to endometrial stromal sarcoma and non specific spindle cell stromal sarcoma.

Immunohistochemically they show positivity for CK7 and calretinin and CD10. PAX-2 is negative. The ductal variant has to be differentiated from endometroid adenocarcinoma, which has intraluminal eosinophilic material. The tubular variant has to be differentiated from mesonephric hyperplasia. This is a a very rare tumor.

There are few rare variants of endocervical adenocarcinoma which are signet ring type, microcystic type, small intestinal type, and adenocarcinoma with hepatoid differentiation and choriocarcinomatous differentiation.

EARLY INVASIVE ADENOCARCINOMA

Early invasive carcinoma is difficult to differentiate from adenocarcinoma in situ.The main differentiating feature is the invasion of the

stroma, which is present in early invasive carcinoma.The invasion may be identified by following features:

Glands with atypia in deeper region, single glands , small and irregular glands, intensive glandular budding, stroma showing desmoplastic reaction with inflammatory reaction, presence of back to back glands, complexely branched papillary architecture. Lymph node metastasis is very rare

ADENOCARCINOMA IN SITU:

A lesion in which normally situated glands are partly or wholly replaced by cytologic malignant epithelium, and the border is characteristically sharp. Intracytoplasmic mucin is absent in the lining epithelium, and looks like endometrial glandular epithelium. Occasionally, the lining epithelium of the glands contain intestinal goblet cells and paneth cells. The neoplastic glands present in the expected location of normal endocervical glands and donot extend beyond the deepest normal crypt. Commonly they have adenoid cystic carcinoma like pattern. The glands are lined by stratified and are arranged perpendicularly to the basal layer. The nucleus is oval, and hyperchromatic with mild pleomorphism and is located basally. Mitosis are common and are present on the luminal side. Apoptosis is prominent. The neoplastic epithelium may affect the surface , where it is often single layered , but more commonly is found in the crypts. The cell types, in order of frequency are endocervical, endometroid and intestinal and

a rare tubal variant. Although the stroma may be intensely inflamed, there is no desmoplastic reaction.

Adenocarcinoma in situ is associated with CIN in at least 50% of cases and is immunoreactive for carcinoembryonic antigen in 80% of cases.

NEUROENDOCRINE CARCINOMA:

Synonym: (atypical) carcinoid tumor, argyrophil cell carcinoma, (extrapulmonary) small cell carcinoma, neuroendocrine carcinoma, and carcinoma with neuroendocrine differentiation. The age distribution of neuroendocrine carcinoma of the cervix and association with HPV[(59)] are the same as for squamous cell carcinoma. The carcinoid syndrome is invariably absent, but some cases have been seen in association with Cushing syndrome, and with inappropriate secretion of antidiuretic hormone (ADH). In contrast to squamous cell carcinoma, CIN changes in the adjacent epithelium are extremely rare. A possible precursor lesion in the form of endocrine cell hyperplasia of the cervix has been identified.The better differentiated tumors have an organoid arrangement, with trabecular, insular, glandular, and spindle patterns of growth. Argyrophilic (but not argentaffin) granules can be demonstrated in many of the cases, particularly the better differentiated tumors. Amyloid may be deposited in the stroma. Ultrastructurally, a variable number of dense-core secretory granules are found in all but the most undifferentiated types. Immunohistochemically, positivity may be found for neuron-specific enolase, CD56,[60]

chromogranin, synaptophysin, 5-hydroxytryptamine (serotonin), other generic neuroendocrine markers,[61] and a variety of peptide hormone. They also commonly express keratin CEA, TTF1 and p16. The large majority of these cervical neoplasms are histologically and clinically aggressive.[62] The prognosis is generally poor.

A definite relationship exists between degree of microscopic differentiation and clinical behavior, the outcome being particularly worse for the small cell carcinomas.There is also a close relationship between clinical stage and prognosis.[63] Both small cell and large cell neuroendocrine carcinomas with a polypoid shape or arising in polyps may be associated with a more favorable prognosis.

HISTOPATHOLOGIC PROGNOSTIC FACTORS:

The histopathologic prognostic factors are clinical staging, size of the tumor, depth of invasion , age of the patient, lymphnode metastases and parametrial extension. In these factors, the clinical staging of the tumor is the most significant one. Histological tumor types does not have significant effects on prognosis.

Staging of disease:

The clinical staging of the disease at the time of diagnosis is an important prognostic factor and it determines the survival of the patient (FIGO STAGING- ANNEXURE 3). In stage I disease, there is difference in survival rate between IA and IB. generally stage I has better survival rate.

About 95% of patients belonging to stage IA has 5-yr survival rate. This percentage gets reduced to about 80% to 90% in patients with stage IB and still gets reduced to about 75% in cases with stage II and still lower to 50% for stage III. Stage IV patients usually presents with local metastasis to adjacent kidney, bladder, urethra, at the time of diagnosis. So the survival of these patients is generally reduced and they die of complication due to local metastasis.

Histological types:

Poorly differentiated carcinoma and small cell carcinoma has bad prognosis than other types. Adenocarcinoma has 10% lower 5 year survival rates than squamous cell carcinoma.

Size of tumor:

Generally tumor of small size has better prognosis than larger tumor. The 5-yr survival rate of the tumors of size less than 3 cm were about 86%. Tumor of size 3-5 cm has 5 yr survival rate of about 76%. For tumors of size larger than 5 cm ,the 5-years survival rates were 61.5%.

Depth of stromal invasion:

It is the distance to which tumor had extended into the stroma. It is measured from the basal layer of the epithelium to the deepest point of tumor extension. When the depth of invasion is more, there is a high chance for the lymphnode metastasis and parametrial extension and recurrence of tumor. The stroma that is free of tumor serves as a barrier[(64)] to the spread of tumor.

Lymphovascular invasion:

The lymphovascular invasion is defined as that the presence of tumor mass within any space lined by endothelial cells and adherence of tumor cells to the endothelial cells. When the depth of invasion is more, there is increased chance for the occurrence of lymphovascular invasion[(65)]. LVSI is associated with decreased survival rate, and increased risk of recurrence.

Parametrial involvement:

The tumor spreads to the parametrium either by contiguous spread and to lesser extent from lymphatic spread. Survival rate of stage I and II cancers is 90% without parametrial involvement, which gets reduced to77% with parametrial involvement. Involvement of parametrium with tumor shows high incidence of lymphnode metastasis, blood vessel invasion, recurrence of tumor and increased mortality.

Nodal status:

The metastasis of lymphnode depends on several factors like clinical staging, size of tumor, distance of stromal invasion, lympho vascular invasion and grade of tumor. As the stage progresses to stage III and IV, there is increased chance of developing lymphnode metastases.The cervical cancer spreads to obturator, paracervical and external iliac nodes. The presence of pelvic node metastasis carries worse prognosis. Rarely there is involvement of para-aortic and scalene lymphnodes and if present it

indicates dissemination of cancer.5 yr survival rate was observed to decreases in the presence of pelvic lymphnode.

AGE OF PATIENT:

Females under 30 yrs of age ,usually found to have better survival than elderly patients. But if they are compared to the clinical staging, there is difference in the survival rate. The younger females have worse prognosis , when they have higher stage at the time of diagnosis[66].

MOLECULAR GENETICS:

In the pathogenesis of cervical carcinoma there are three major components, two of them related to the role of human papillomaviruses (HPV). First, the effect of viral E6 and E7 proteins. Second, the integration of viral DNA in chromosomal regions associated with well known tumor phenotypes. Some of these viral integrations occur recurrently at specific chromosomal locations, such as 8q24 and12q15, both harbouring HPV18 and HPV16. And third, there are other recurrent genetic alterations not linked to HPV. Recurrent losses of heterozygosity (LOH) have been detected in chromosome regions 3p14–22, 4p16, 5p15, 6p21–22, 11q23, 17p13.3 without effect on p53,18q12–22 and 19q13, all of them suggesting the alteration of putative tumor suppressor genes not yet identified. Recurrent amplification has been mapped to 3q+ arm, with the common region in 3q24–28 in 90% of invasive carcinomas. The mutator phenotype, microsatellite instability, plays a minor role and is detected in only 7% of

cervical carcinomas. The development of cervical carcinoma requires the sequential occurrence and selection of several genetic alterations. The identification of the specific genes involved, and their correlation with specific tumour properties and stages could improve the understanding and perhaps the management of cervical carcinoma.

The LOH means that a particular DNA region has been lost, and therefore, if this loss is recurrent, then it is considered significant. LOH is generally thought of as an intermediate step in the inactivation of tumour suppressor genes, such as p53or RB. Inactivation of TP53 appears to play a key role in the development of malignancy in HPV infection.

TP53 inactivation is important in the progression from intraepithelial to invasive neoplasia[67-69]. Its reactivity is seen in both in situ lesions and invasive adenocarcinoma. It is absent in villoglandular adenocarcinoma and in minimal deviation adenocarcinoma.

FHIT (fragile histidine triad) gene abnormality , including loss of heterozygosity, homozygous deletions and aberrant transcripts , are common in cervical carcinomas. This gene abnormalities have been found in both CIN and invasive carcinoma.

Monoclonality is seen in early invasive carcinoma and in nearly all cases of high grade CIN found to be monoclonal.

Recurrent amplifications and chromosome gain:

Gene amplification and chromosome gain are two different mechanisms by which a tumour cell can increase a gene dosage. It has been found that fivefold or more amplifications were found for MYCL1, SEA,CCND1,BCL1and GLI genes, HRAS and HER2/neu. H ER2/ neu cases also showed rearrangement of the 17q11.2–12 band, suggesting a possible damage of this gene. Overexpression of ERBB2 gene has been previously reported in 60% of the cases studied (Pinion et al, 1991).ERB2/neu amplification has been observed in other carcinomas, including breast and ovary (Mitra et al, 1994b)

Prognostic biomarkers:

1. Ki 67

Ki-67 is a proliferative marker .In normal conditions,it is usually present in the supra basal cells, overlying the basal cells of the normal cervical epithelium. If this marker is expressed in the superficial layer of cervix, it indicates presence of infection of HPV and related intraepithelial lesion. This is expressed in LSIL, HSIL and carcinoma. Increased expression of Ki-67 is associated with poor prognosis.

2. p16:

p16 is an inhibitor of kinase which is cyclin-dependent. This marker is highly reliable and its expression is related to infection caused by HPV of high risk and intermediate risk. This is absent in low risk HPVinfection.

1. Strong positive staining is seen in HPV infection produced by intermediate group and high risk strain
2. Weak staining is seen in epithelium with mild atypia
3. Staining in superficial cells and koilocytes is seen in HPV of low risk type.
4. Focal strong staining is seen in columnar epithelium lining the endocervix

3. COX-2

Cycloxygenase 2 (COX-2) is an enzyme that is involved in the formation of prostaglandins from arachidonic acid. It is expressed in angiogenesis and thus increased in metastasis. In carcinoma cervix , the expression of COX-2 indicates poor prognosis[70].In HPV- 16 infection ,E6 and E7 oncoprotein through epidermal growth factor receptor regulates the transcription of COX-2

4. CD 31

It is an angiogenesis marker and its increased expression is associated with poor prognosis.

5. HER-2-Neu

HER2neu is the human epidermal growth factor receptor 2. This marker is found to be expressed in many tumors including breast,endometrium, ovary, stomach, oesophagus and cervical cancer. It indicates progression of tumor and is usually associated with poor prognosis[71]. In studies , it was found that HER2 was expressed in varying

degrees upto 76% of cervical cancers. Many clinical trials have been done using HER2 inhibitors to cancer cervix patients. In recent studies, it was found thatHER2 expression did not correlate with the response of tumor , when treated with EGFR inhibitor.

6. D2-40 positive

Endothelial cells of lymphatic vessels express this marker .By counting D2-40 positive vessels , we can assess the lymphatic vessel density in the tumor. It has been observed that high expression of this marker is related to lymphnode metastasis and high tumor stage and thus indicates poor prognosis in cervical cancers[72].

7. C-myc expression

8. LRIG 1

Leucine rich repeats and immunoglobulin like domainsI(LRIG1) restricts signaling of growth factor by increasing ubiquitylation and also by EGFR degradation .It also inhibits the action of oncogenes Met or Ret.

The expression of LRIGI indicates favourable prognosis[73], as it inhibits the growth factors and thus limiting the tumor progression.

9.**CD 4 positive lymphocytes**

Infiltration of CD4 positive lymphocytes of tumor cells indicates better survival. Its expression is associated with good prognosis.

LRIG-1 and CD-4 lymphocytes are indicators of good prognosis, if they are present. All others are poor prognostic factors Cyclin- E is an another marker that indicates infection by human papilloma virus.

IMMUNOHISTOCHEMISTRY:

IHC is a technique in which the antigen of interest in the particular tissue is detected by recognition of antigen- antibody complex. Progression of the tumor makes it more poorly differentiated, and the categorization of the tumor into specific types by histopathological techniques becomes very difficult. These tumors are histologically classified as undifferentiated, anaplastic , or small cell carcinoma. For clinicians ,it is very important to say whether the tumor belongs to epithelial, mesenchymal or of lymphoid cell of origin[74] ,as their mode of treatment varies depending on the diagnosis.

Immunohistochemistry is referred to as brown revolution. Immunohistochemistry helps the pathologists to fit the tumor, that has been classified as undifferentiated in histology , into a specific histogenetic origin.The subtyping of tumor is essential as it indicates prognosis and also thereby direct the clinicians to a targeted therapy against the particular tumor[75]. To treat the patient adequately, it is essential to diagnose histologically and to categorize the tumor into its subtype. Histological diagnosis becomes difficult in certain tumors because of its varying presentation and undifferentiated feature in histology. Such tumor accounts for about 5-10% of all diagnosed tumors[76]**.** In such malignancies , advanced recent technologies play an important role. Among such technologies, immunohistochemistry is the method commonly utilized.

Immunohistochemistry identifies the particular antigen of interest ,for example HER2, or p53 ,by the formation of antigen antibody complex.It

seeks to exploit the specificity provided by the binding of an antibody with its antigen at a light microscopic level. IHC has a long history, extending more than half a century from 1940, when Coons developed an immunofluorescence technique to detect corresponding antigens in frozen tissue sections.[77]

IHC has been used for the identification and demonstration of both prognostic and predictive markers. The basics of IHC are similar to that of histochemistry and it is not an alternative for histochemistry but actually it act as a valuable adjunct. As emphasized by pioneers in this field of functional morphology, "the object of all staining is to recognize microchemically the existence and distribution of substances which we have been made aware of macrochemically[78].

The basic critical principle of IHC, as with any other special staining method, is a sharp visual localization of target components in the cell and tissue, based on a satisfactory signal-to-noise ratio. Amplifying the signal while reducing non-specific background staining (noise) has been a major strategy to achieve a satisfactory result that is useful in daily practice.

Antibody molecules are proteins; thus any rigid part of an antibody molecule may itself serve as the antigenic determinant to induce an antibody. IHC techniques exploit the fact that immunoglobulin molecules can serve both as antibodies and as antigens . Evaluation of an antibody for use in IHC

is based on two main points: the sensitivity and the specificity of the antibody-antigen reaction for IHC.

The development of the hybridoma technique provided an almost limitless source of highly specific antibodies. Monoclonal antibodies do not guarantee antigen specificity; however, since different antigens may share similar or cross-reactive epitopes, the “practical” specificity reflected by IHC is excellent for most monoclonal antibodies. In contrast, a “polyclonal antibody” is an antiserum that contains many different molecular species of antibody having varying specificities against the different antigens (or antigenic determinants) used to immunize the animal.

It is important to remember that polyclonal antibodies may also include varying amounts of antibodies to a whole range of antigens (including bacteria and viruses) that the immunized animal encountered before its use as a source of antibody. As a result, polyclonal antibodies often give more non-specific background staining in slides than encountered using monoclonal antibodies.

Comparison of sensitivity and specificity between polyclonal and monoclonal antibodies indicates that polyclonal antibody may be more sensitive but less specific than monoclonal antibody. The reason may be that polyclonal antibody (actually a composite of many antibodies) may recognize several different binding sites (epitopes) on a single protein

(antigen), whereas a monoclonal antibody recognizes only a single type of epitope.

Background staining of tissues that may be due to nonspecific antibody binding or to the presence of endogenous enzymes. Background staining should be blocked to get good results. Non-specific antibody binding is due to polyclonal antibody, because multiple “unwanted” antibodies may exist in the antiserum. Preincubation with normal serum usually reduces these kinds of non-specific binding.Blocking endogenous enzyme activity is also important. Any residual activity of these endogenous enzymes must be abolished during immunostaining in order to avoid false-positive reactions Peroxidase activity is present in a number of normal and neoplastic cells, including erythrocytes, neutrophils, eosinophils, and hepatocytes.

When performing an immunohistochemical study in tissues rich in blood cells, such as bone marrow, it is recommended that a“peroxidase-blocking” step be used, coupled with a“substrate control” (i.e., a section treated only with the hydrogen peroxide–chromogen mixture to visualize the extent of endogenous peroxidase activity).

Otherwise, alternative methods, such as alkaline phosphatase, glucose oxidase, or immunogold labeling, may be used to avoid the possibility of confusion with any endogenous enzyme activity.

The blocking of endogenous enzymatic activity must be carried out before the addition of enzyme-labeled secondary reagent; otherwise, the enzyme label is also inactivated by the blocking procedure, resulting in a false- negative result. Antibody molecules cannot be seen with the light microscope or even with the electron microscope unless they are labeled or flagged by some method that permits their visualization. Essentially, detection systems attach certain labels or flags to primary or secondary antibodies in order to visualize the target antibody antigen localization in the tissue sections. A variety of labels or flags have been used, including fluorescent compounds and active enzymes that can be visualized by virtue of their property of inducing the formation of a colored reaction product from a suitable substrate system. The detection includes following methods like direct conjugate – labeled antibody method, indirect or sandwich procedure, unlabeled antibody methods which includes enzyme bridge techniques, peroxidase-antiperoxidase method, biotin- avidin method, Avidin –biotin conjugate procedure, biotin-strepavidin system.

Other methods include Alkaline phosphatase label, double stains, & polyvalent detection system which has alkaline phosphatase –antialkaline phosphatase method, and polymer based labeling , basic 2 step method, tyramine signal amplification method, titration of primary antibody and detection method.

After detection of antigen-antibody complex, amplification is done. This is classified into three methods: Pre amplification, Detection amplification, Post detection amplification.

Pre amplification includes antigen retrival. Antigen retrival is done by applying high temperature, formalin fixed antigen is retrieved. Following techniques are

Used:

1. Water bath methods
    A. conventional water bath heating
    B. Dako PT link (Automated)
2. Pressure cooker heating
3. Autoclave heating
4. Microwave oven heating
5. Proteolytic pretreatment
6. Combined proteolytic pretreatment & HIER(heat induced epitope retrieval)
7. Combined deparaffinisation & target retrieval.

The composition and PH of the buffer are crucial for optimal retrieval.Commonly used buffer is citrate buffer . Other high PH buffer are also being used.

Detection amplification is by

A. Multistep detection system- PAP, ABC, APAAP, BSA

B. Stepwise amplification

C, Polymeric and polylabelling amplification

Post detection amplification is by

A. Enhanced DAB by metal, imidazole & sba on CARD

B. Anti- end product

C. Gold/silver enhancement method

After this , chromogen (colour producing substrate system) is added to impart colour to the antigen antibody complex. Usually DAB is used, with horseradish peroxidase and gives brown colour.

Then counterstain with hematoxylin and mounting as usual routine procedure.

There are standardized procedures for immunohistochemical staining,laid by Ad- Hoc committee.

Recommendations for Improved Standardization of immunohistochemistry by the Ad-Hoc Committee110 Recommendation.

1. Fix all specimens promptly in 10% neutral buffered formalin.
2. Fix and process resections and core biopsies in an identical manner.
3. Only use 10% neutral phosphate buffered formalin.
4. Note that fixation is 8 to 72 hours for both core biopsies and resections.
5. Use formalin, not alcohol, to fix cytology specimens for ER assay.

6. Use conventional tissue processors to process breast tissue.
7. Ensure that the first formalin containers on the tissue processor are always newly replenished.
8. Ensure that tissue processor fluids do not exceed 37°C.
9. Be sure that paraffin in the tissue processor does not exceed 60°C.
10. Record and document fixation times in your report.
11. Use *in vitro* diagnostic kits that employ clone, 6F11, 1D5, or SP1.
12. Include positive and negative controls with each batch run.
13. Employ a threshold for positive result of 1% positive cells.
14. Report semi-quantitation, tabulating the intensity (0, 1+, 2+, 3+) and percent of positive cells.

In this study, Her2 neu is taken as prognostic marker in cervical carcinoma, which is identified by immunohistochemistry.

HER 2 NEU:

HER2 is the Human EGFR-2, which belongs to HER family. The gene encoding HER2 is located on chromosome 17 and is a member of EGF/erbB growth factor receptor family.

The four members of the human epidermal growth factor receptor (HER) family: HER1 (EGFR, erbB1), HER2(erbB/neu), HER3 (erbB3) and HER4 (erbB4) are transmembrane tyrosine kinases, type I receptor, which take part in the regulation of cell proliferation, adhesion, migration and differentiation [(79)]

Receptor activation and signal transduction act through homo- and heterodimerisation. The formation of various dimeric pairs is dependent on the affinity between the different receptors and the concentration of both ligands and receptors[80]. The specific pattern of the heterodimeric pairs seems to modulate the intracellular response. Over expression of HER2 recptors results in receptors transmitting excessive signals for cell proliferation to nucleus. This may lead to more aggressive growth of the transformed cell. Data supports the hypothesis that the HER2 transformed cells directly contribute to the pathogenesis & clinical aggressiveness of tumor that overexpress HER2.

Receptor overexpression, especially of HER1 and HER2 has been associated with malignant potential and poor prognosis in various tumors[81].

These findings led to the development of specific antibodies targeting HER1 and HER2 for modern anticancer therapies . The development of pan HER inhibitors are hoped to further enhance the anti-tumor effects. In breast cancer as well as in other tumors, the anti-HER2 antibody trastuzumab (HerceptinTM,R) significantly improves survival, the strong anti-tumor activity being restricted to HER2-overexpressing tumours .

In carcinoma cervix, there are conflicting results regarding the frequency of HER2 expression, its prognostic consequence and consequently its value as a potential therapeutic target. Regarding the role of the other EGF receptors in cervical cancer, even more controversies exist. The aim of this study, is to investigate the expression pattern of HER2 and the influence on prognosis ,focusing on carcinomas of the uterine cervix.HER2 Protein over expression is determined by membrane staining intensity and the slide evaluation should be performed using light microscope.

TABLE no:3 HER2 scoring is done as follows:

Score to report	**HER2 expression assessment**	**Staining pattern**
0	Negative	No staining is observed or membrane staining is observed in <10% of tumor cells
1+	Negative	Faint / barely perceptible membrane staining is detected in >10% of tumor cells. The cells exihibt incomplete membrane staining
2+	Weakly positive (equivocal)	Weak to moderate complete membrane staining is observed in>10% tumor cells
3+	Strongly positive	A strong complete membrane staining is observed in > 10% of tumor cells

MATERIALS AND METHODS

STUDY LOCATION:

The study was conducted at Department of Pathology, Tirunelveli Medical College.

STUDY PERIOD:

The study was conducted prospectively from 2012 to 2014.

SAMPLES:

INCLUSION CRITERIA:

1. Cervical biopsies diagnosed as carcinoma cervix.
2. Hysterectomy specimen diagnosed as carcinoma cervix.

EXCLUSION CRITERIA:

1. Other cervical biopsies diagnosed as Inflammatory conditions, benign Lesion.
2. Cervical biopsies diagnosed as carcinoma in situ and mesenchymal lesion.
3. Hysterectomy specimens of causes other than carcinoma cervix.

SAMPLE SIZE:

100 cases of carcinoma cervix. [Annexure-6]

METHODOLOGY:

DATA COLLECTION:

The data of patients age, complaints, clinical staging, and other clinical data including lymphnode status and parametrial extensions are obtained from the pathology records and from case sheet of the patients. The data are recorded in a format(Annexure 1)

PROCESSING OF SPECIMEN:

Cervical biopsy specimen and hysterectomy specimens received were fixed in 10% formalin and processed routinely.

GROSSING:

Biopsy specimens were presented in toto and in hysterectomy specimen, cervix was carefully sectioned to include endocervix and ectocervix. Sections of 4-5µ thickness were cut and stained with Haematoxylin & Eosin

STAINING TECHNICQUE:

Annexure4

The slides are studied under light microscopy and the data are recorded. Then the sections are subjected to Immunohistochemical staining.

IMMUNOHISTOCHEMICAL EVALUATION:

Immunohistochemistry is performed on 3-4m-thick sections taken on poly-L-lysine-coated slides. Antigen retrieval was performed by heating the sections in citrate-buffer at pH 6.0 using pressure cooker. Rabbit Monoclonal

antibody (Thermo Fisher Scientific Laboratories) is used to bind with primary antigen and is detected by adding secondary antibody conjugated with horse radish peroxidase – polymer and diaminobenzidine substrate. In this study , HER2neu antigen of Thermofisher laboratory products is used.

PROCESSING FOR IMMUNOHISTOCHEMISTRY

- 3µm thickness sections were cut using microtome from the selected paraffin blocks.
- The sections are taken in poly L-lysine coated adhesive slides.The slides are incubated at 60 c for 1 hour
- The slides are subjected to 2 changes of xylene , 5 minutes each for deparaffinization.
- They are then transferred to absolute alcohol for 5 minutes followed by 80% and 70% alcohol for 5 minutes to rehydrate the tissue sections.
- Tissue sections are then placed in running tap water for 5 minutes and washed in distilled water
- Antigen retrieval was performed using pressure cooker in citrate buffer
- Then the sections are cooled to room temperature and the slides are washed with distilled water

- Endogenous peroxidase activity is removed by incubating the tissue sections with enough drops of 3% peroxide block in a humidity chamber for 5minutes.The sections are then washed in TRIS wash buffer.
- Primary antibody (Her2 neu) is then added over the tissue sections and incubated for 30 minutes .
- The tissue sections are then washed in TRIS wash buffer.
- Followed by that primary amplifier is added for 15 minutes to enhance the process of primary antibody which is then washed in TRIS wash buffer
- Secondary antibody is added and incubated for 20 minutes and then washed with TRIS wash buffer
- DAB chromogen (1ml DAB buffer +1 drop DAB chromogen) is then added over the tissue and incubated for 4 minutes and then washed with 2 changes of distilled water.
- Counterstaining was done with haematoxylin for 30 seconds and washed in running tap water.
- Dehydration is done by 2 changes of 100 % alcohol.
- Mounting is done by DPX mountant and observed under microscope.

BUFFER PREPARATIONS

1. Citrate buffer

Citric acid	-	1.92 gms
Distilled water	-	1000ml

Ph is adjusted to 6.2 with 1N NaoH

2. Tris wash buffer

Tris	-	0.605 gm
Sodium chloride	-	8 gm
1 N Hcl	-	4ml
Distilled water	-	1000 ml

PRECAUTIONS

1. The glassware's used should be dry and clean.
2. All the buffers used should be prepared fresh and the p H should be adjusted according to the preferred p H.
3. The staining procedures are never allowed to dry so they are performed under a humidity chamber.
4. DAB chromogen should be handled and disposed carefully as it is a carcinogen.
5. Primary,secondary antibody, DAB chromogen, peroxidase block, Amplifier,everything should be stored at 4-6ºC

While performing IHC every batch should have a positive control slide. Then the slides are counterstained with Mayers Hematoxylin.

SCORING OF HER2/neu STAINING:

Her2/neu positivity is graded as per ASCO guidelines.A positive reaction was taken as crisp golden brown membranous and cytoplasmic staining. Intensity of staining was graded under ASCO scoring system as strong, complete membrane staining in more than 10% of malignant cells (3+); week to moderate complete staining in more than 10% of malignant cells (2+); no or fewer than 10% cells staining (0 to 1+) respectively.

STATISTICAL ANALYSIS:

Statistical analysis of data was performed using statistical package for social science software version 11.5 . HER2/neu expression and its correlation with various factors was calculated using Pearsons Chi-square test. *P* value <0.05 was taken as statistically significant.

ETHICAL CONSIDERATION:

The study was conducted after obtaining approval from the Institutional Ethical Comittee of Tirunelveli Medical College, Tirunelveli. The study included cervical biopsies and hysterectomy specimens submitted for histopathological examination to the Department of Pathology, Tirunelveli Medical College from 2012 to 2014.

OBSERVATION AND RESULTS:

Study design:

A total of 100 cases of carcinoma of cervix obtained as either cervical biopsies or hysterectomy specimens were included in the study. Among these 100 cases, 75 cases were squamous cell carcinoma(75%) , 21 cases were adenocarcinoma(21%), 3 cases were small cell carcinoma(3%), 1 was adenosquamous carcinoma.

Table 4: Distribution of histological types of carcinoma cervix:

Types of carcinoma	Total no of cases(n=100)
Squamous cell carcinoma	75
Adenocarcinoma	21
Small cell carcinoma	3
Adenosquamous carcinoma	1

IHC for HER2/neu oncoprotein was done in these 100 cases.

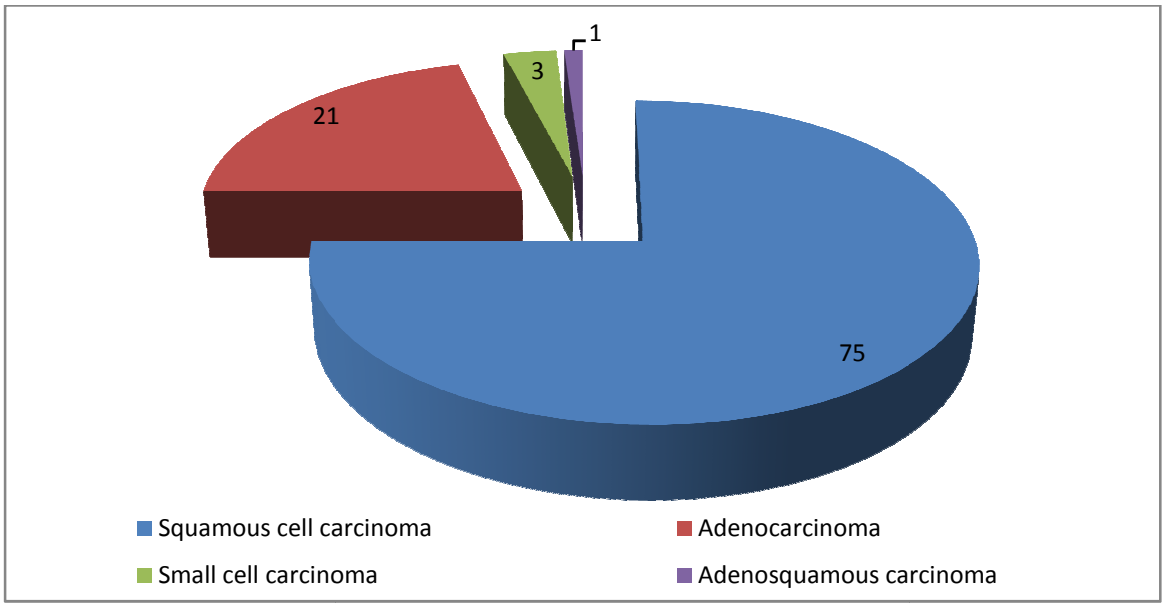

Chart no 1: Distribution of carcinoma cervix cases among histological types

This chart no 1 shows the distribution of cases between histological types of carcinoma cervix. Among 100 cases, 75 were squamous cell carcinoma, 21 were adenocarcinoma, 3 were small cell carcinoma, and 1 was adenosquamous carcinoma.

AGE WISE DISTRIBUTION OF CASES:

Table 5: Distribution of cases according to age group in carcinoma cervix

Age group of patients	Number of cases(n=100)
30-40yrs	11
41-50yrs	22
51-60yrs	30
61-70yrs	30
71-80yrs	7

When cases were categorized based on age group,30 cases fall under 51-60yrs of age, another 30 cases were of 61-70 yrs, 22 cases were of 41-50 yrs, 11 cases fall under 30-40 yrs, 7cases were of 71-80yrs.

Table 6: Distrbution of HER2/neu expression among age wise distribution of cases of carcinoma cervix:

Age group	No of HER2 positive cases(n=17)
30-40yrs	0
41-50yrs	5(22.7%)
51-60yrs	6(20%)
61-70yrs	5(16.6%)
71-80yrs	1(14.2%)

DISTRIBUTION OF HER2/neu EXPRESSION IN CARCINOMA CERVIX:

Among 100 cases of carcinoma cervix, HER2/neu expression is seen in 17 cases. Out of this 17 cases , 16 cases are squamous cell carcinoma and 1 case is of small cell carcinoma.

Table 7: Distribution of HER2/neu expression among histological types of carcinoma cervix

Type of carcinoma	No of HER2/neu positive cases (n=17)
Squamous cell carcinoma	16(94.11%)
Small cell carcinoma	1(0.05%)
Adenocarcinoma	0
Adenosquamous carcinoma	0

SQUAMOUS CELL CARCINOMA:

In 75 cases of squamous cell carcinoma, there were 23 cases of well differentiated carcinoma (30.6%),48 cases were(64%) moderately differentiated, and 4 cases were(5.33%) poorly differentiated carcinoma. These data are mentioned in the following Table 8

Table 8: Distribution of Histological grades of Squamous cell carcinoma:

Grades of squamous cell carcinoma(n= 75)	Number of cases
Well differentiated carcinoma	23(30.6%)
Moderately differentiated carcinoma	48(64%)
Poorly differentiated carcinoma	4(5.33%)

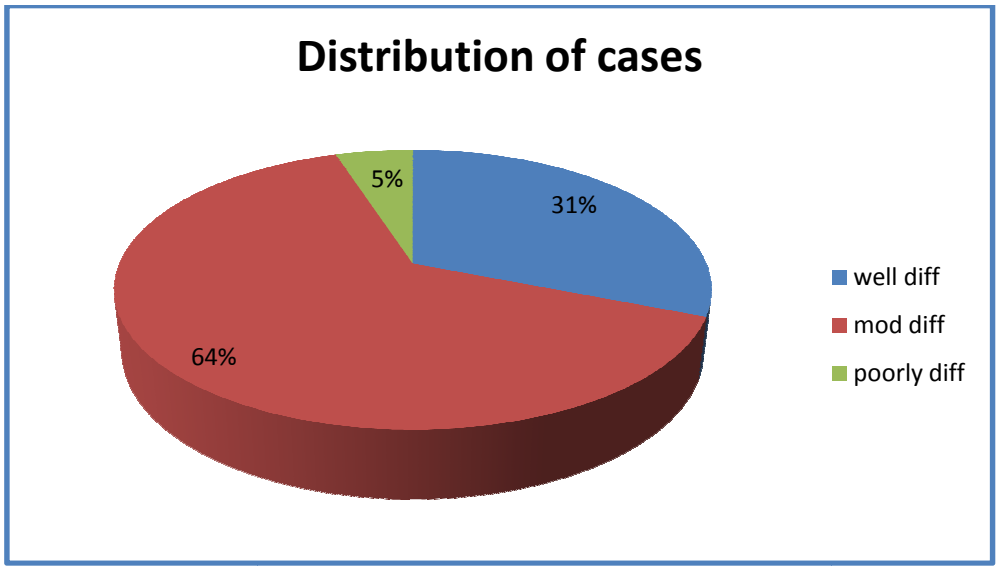

Chart no:2 Distribution of cases among grades of squamous cell carcinoma

Among 75 cases of squamous cell carcinoma , 16 cases(21.33%) of squamous cell carcinoma were positive for HER2/neu oncoprotein. Among HER2 positive squamous cell carcinoma, 12 cases(25%) were of moderately differentiated, 3 cases(13%) were of well differentiated, 1 case (25%) of poorly differentiated carcinoma. The data are given in the following Table 9

Table 9: Distribution of HER2neu expression in grades of squamous cell carcinoma.

Grades of squamous cell carcinoma	HER2 positive cases (n=16)	Percentage
Well differentiated carcinoma(n=23)	3	13.04%
Moderately differentiated carcinoma(n=48)	12	25%
Poorly differentiated carcinoma(n=4)	1	25%

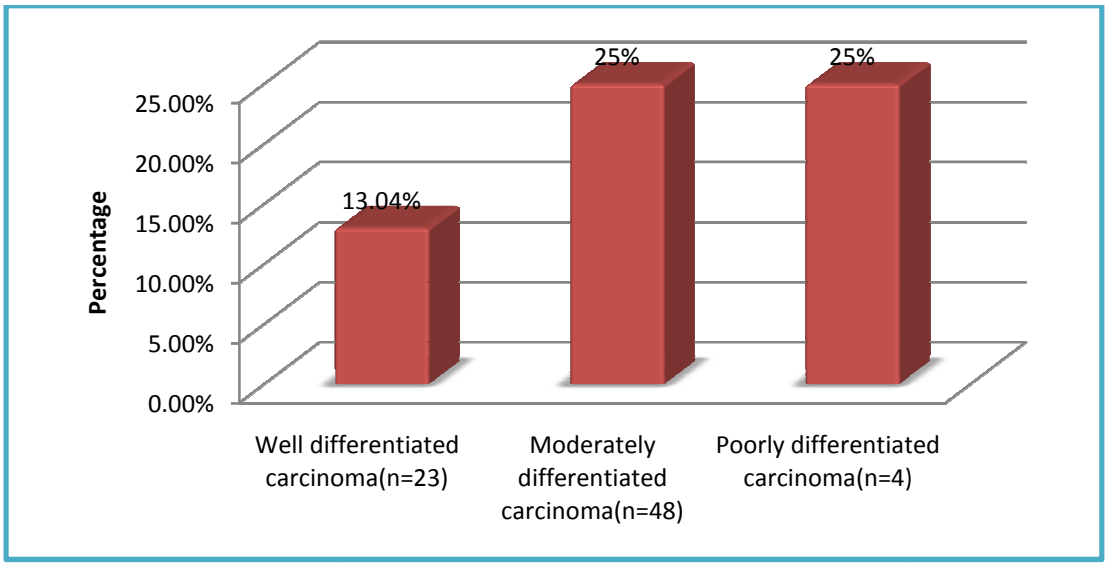

Chart no:3 Distribution of HER2neu expression among grades of squamous cell carcinoma

GRADING OF HER2/neu SCORE IN SQUAMOUS CELL CARCINOMA

Table 10 : Grading score of HER2/neu staining in squamous cell carcinoma:

Grading score of HER2 in squamous cell carcinoma	Number of cases(n=75)
Negative	35(46.6%)
1+	24(32%)
2+	8(10.6%)
3+	8(10.6%)

Among 75 cases of squamous cell carcinoma, 35 cases were negative for HER2/neu (46.6%), 24 cases scored 1+(32%), 8 cases were 2+(10.6%), 8 cases were 3+(10.6%).(details in Table 10)

Table 11: Correlation of HER2/neu expression with grades of squamous cell carcinoma

Grades of squamous cell carcinoma	**HER2 positive cases (n=16)**	**HER2 negative cases**
Well differentiated (n=23)	3(13.04%)	20
Moderately differentiated (n=48)	12(25%)	36
Poorly differentiated (n=4)	1(25%)	3

Chi square test = 1.3584 **; P value is 0.50702.The result is not significant** From the above table , it is undersood that there is no significant correlation of HER2/neu expression with histological subtypes of squamous cell carcinoma. HER2/neu expression is about 13% in well differentiated type, 25% in moderately differentiated type and 25% in poorly differentiated type.

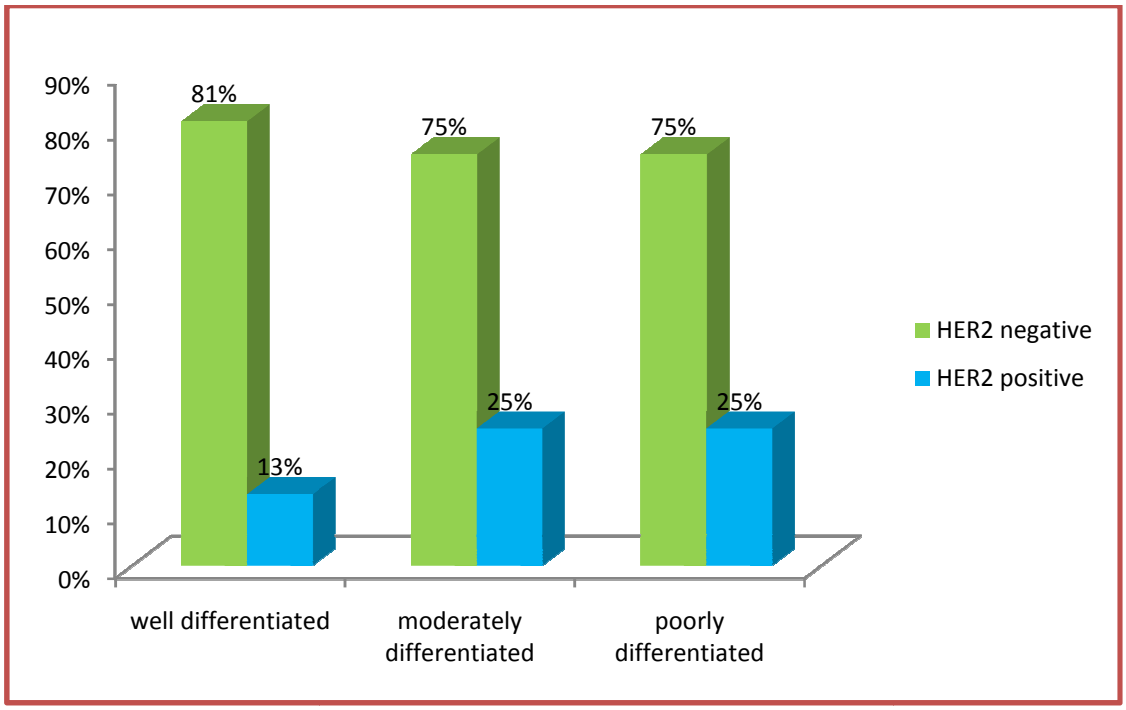

Chart no 4: Distribution of HER2/neu positive cases among histological grades of squamous cell carcinoma

SMALL CALL CARCINOMA:

Among 100 cases, 3 cases were small cell carcinoma. In this 3 cases, 1 case was positive for HER2 /neu protein(33.33%) and score was 2+.

Table 12 : Distribution of small cell carcinoma with its HER2/neu expression and scoring:

Type of carcinoma	Total no of cases	HER2 positive cases	Score of HER2
Small cell carcinoma	3(3%)	1(33.33%)	2+

ADENOCARCINOMA:

Among 100 cases, 21 cases were adenocarcinoma. In this 21 cases , HER2/neu was 1+ in 1 case and all other cases scored negative. According ASCO guidelines, 1+ was considered as negative. So , as a whole Adenocarcinoma case were negative for HER2 /neu expression.

Table 13: Distribution of Adenocarcinoma with its HER2/neu expression and scoring:

Type of carcinoma	Total no of cases	HER2 positive cases	Score of HER2
Adenocarcinoma	21	0	Negative

ADENOSQUAMOUS CARCINOMA:

Among 100 cases, only 1 was adenosquamous carcinoma . This one case was negative for HER2 /neu expression.

Table 14: Distribution of Adenosquamous carcinoma with its HER2/neu expression and scoring:

Type of carcinoma	Total no of cases	HER2 positive cases	Score
Adenosquamous carcinoma	1	0	Negative

STAGING OF CARCINOMA:

According to FIGO STAGING(Annexure 3), 100 cases were categorized as follows:

Table 15: Distribution of cases among clinical staging in carcinoma cervix:

FIGO Stage	No of cases(n=100)
Stage I	64
Stage II	22
Stage III	14

In this 100 cases, 17 cases were positive for HER2 /neu marker. Among this 6 cases of stage I(9.37%) were positive , 4 cases of stage II(18,18%) were positive and 7 cases of stage III(50%) were positive.

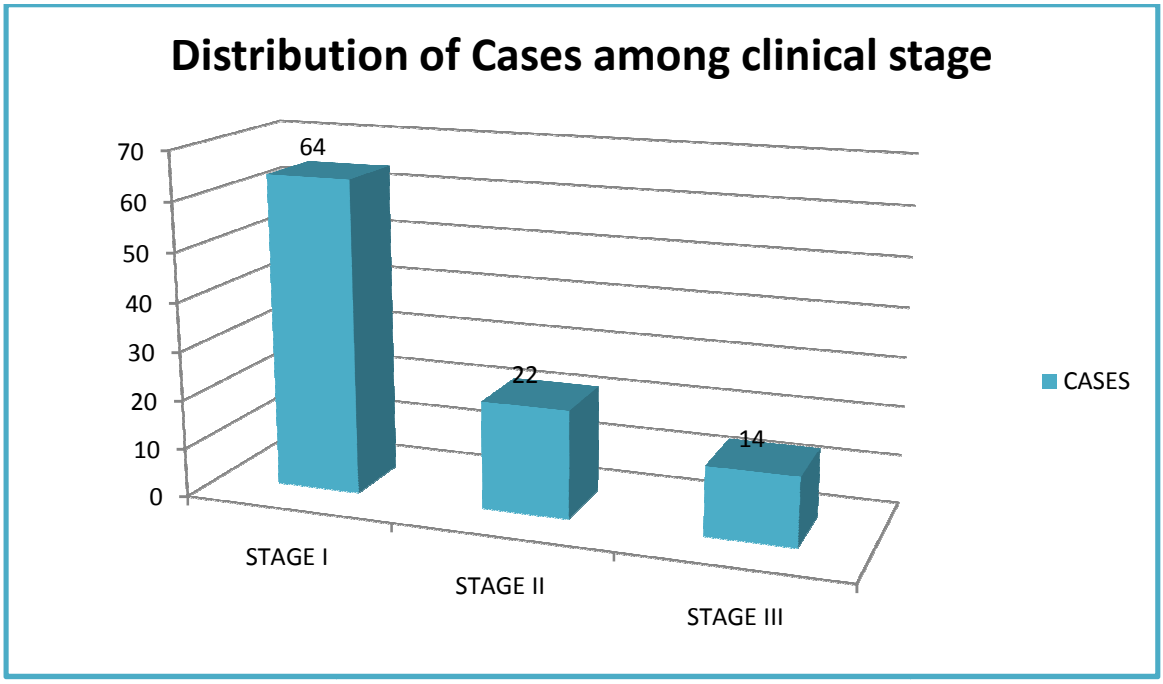

Chart no:5 Distribution of cases of cancer cervix among clinical stage

Table 16: Distribution of HER2/neu expression in clinical staging of carcinoma cervix:

Clinical staging	Total cases	HER2 positive cases(n=17)
Stage I	64	6(9.37%)
Stage I	22	4(18.18%)
Stage III	14	7 (50%)
Stage IV	0	0

Table 17: Correlation of HER 2/neu expression with clinical staging of carcinoma cervix

Clinical staging	Total cases	HER2 positive cases(n=17)	HER 2 negative
Stage I	64	6(9.37%)	58
Stage II	22	4(18.18%)	18
Stage III	14	7(50%)	7
Stage IV	0	0	0

CHISQUARE VALUE – 13.464 ; **P VALUE – 0.0011**

The result is significant

Table no:17 shows the distribution of HER2/neu positive cases in relation to various clinical staging. From the above table it is clear that, stage

III cases had increased HER2/neu expression of about 50% positivity .Thus there is a strong correlation exist between HER2/neu expression and clinical staging.

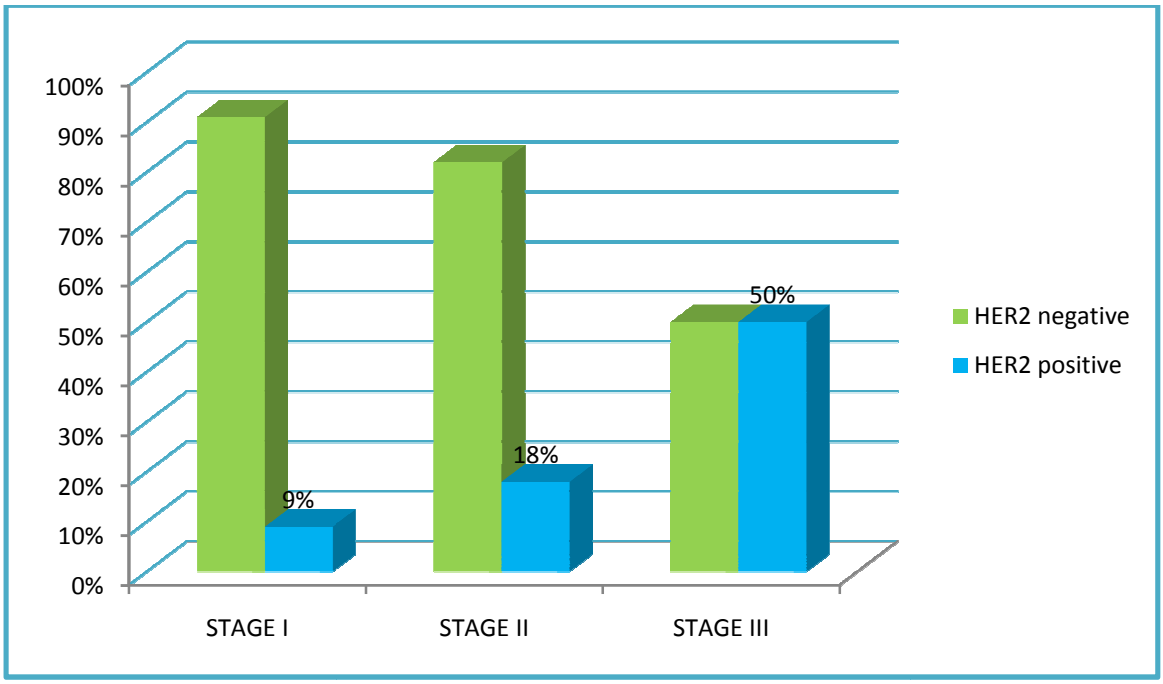

Chart no:6 Association of HER2 /neu positivity with clinical staging of cancer cervix.

It shows the HER2/neu expression increase as the staging of cancer increases.

LYMPHNODE METASTASES:

Among 100 cases , there were 9 cases showed lymphnode metastases. 9 cases were of squamous cell carcinoma only.1 case was well differentiated carcinoma, 7 cases were of moderately differentiated carcinoma and 1 case was of poorly differentiated carcinoma.

Table 18: Distribution of lymphnode metastasis in carcinoma cervix:

Type of carcinoma	Lymph node positive cases(n=9)
Squamous cell carcinoma	9(100%)
Adenocarcinoma	0
Adenosquamous carcinoma	0
Small cell carcinoma	0

Table 19: Distribution of Lymphnode metastasis in squamous cell carcinoma of cervix:

Lymphnode metastases in grades of squamous cell carcinoma	No of cases(n=9)
Well differentiated carcinoma	1(11.11%)
Moderately differentiated carcinoma	7(77.7%)
Poorly differentiated carcinoma	1(11.11%)

Out of the total 100 cases , 9 cases were positive for lymph node metastasis , and out of which 5 cases were positive for HER2 /neu oncoprotein (55.55%) and all of them belonged to moderately differentiated squamous cell carcinoma

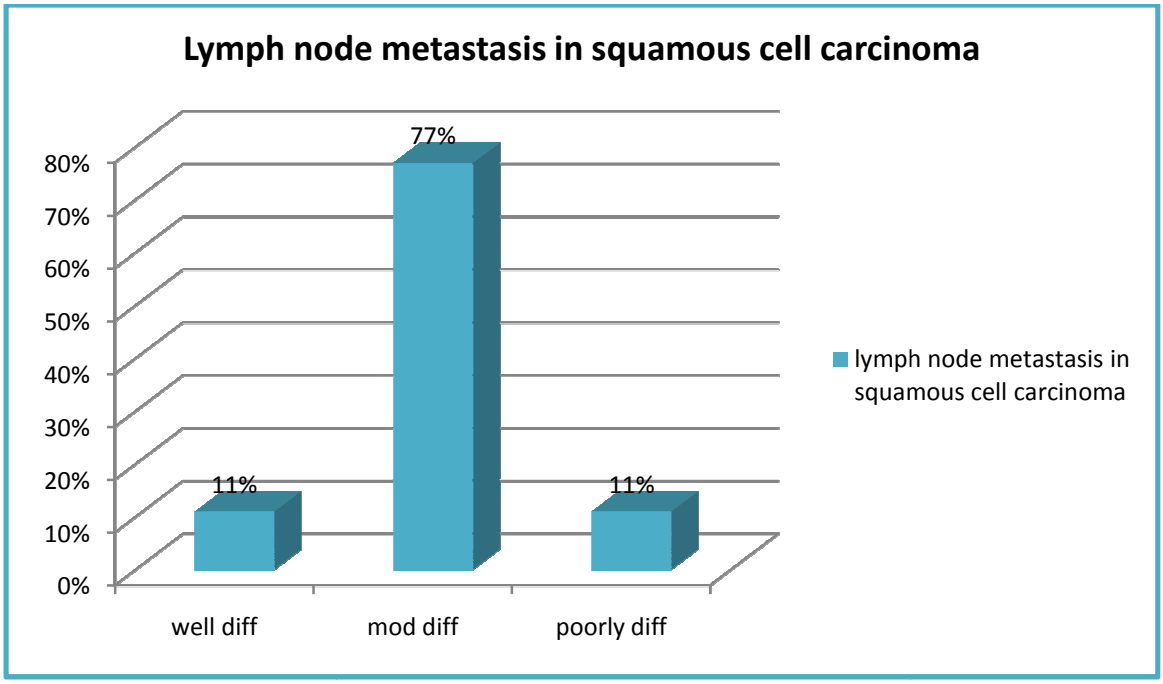

Chart no :7 Percentage of Lymph node metastasis in squamous cell carcinoma

Table 20: Correlation of HER2/neu expression with Lymphnode metastasis in carcinoma cervix:

Lymphnode metastasis	Total cases	HER2 Positive cases	HER2 negative cases	P value
Present	9	5(55.55%)	4	<0.05
Absent	91	12(13.1%)	79	

CHI SQUARE TEST- 13.379 ; **P VALUE – 0.001. The result is significant as P< 0.05**

The above Table no:20 shows the association of HER2 /neu expression with lymphnode metastases . Totally there were 9 cases with positive lymph node metastases. Among this 9 cases , 5 cases showed HER2/neu expression(55.55%).

Based on Pearsons chisquare test, P value is significant and so there is correlation exist between HER2/neu expression and lymph node metastasis in carcinoma cervix.

As all the 5 lymphnode positive cases belonged to squamous cell carcinoma, moderately differentiated type, the association of HER 2/neu positivity in squamous cell carcinoma in lymph node metastasis was studied and correlated.

Table 21: Correlation of HER2/neu expression with lymph node metastasis in squamous cell carcinoma in cervix:

Lymphnode metastasis	Total cases	HER2positive	HER2 negative	P value
Present	9	5(55.55%)	4	<0.05
Absent	66	11	55	

CHI SQUARE TEST- 7.1372 ; **P VALUE – 0.007. The result is significant as P< 0.05**

The above Table no:21 shows the association of HER2 /neu expression with lymphnode metastases in squamous cell carcinoma . Totally there were 9 cases with positive lymph node metastases. Among this 9 cases of squamous cell carcinoma , 5 cases of moderately differentiated carcinoma showed HER2/neu expression(55.55%). As the p value is significant, there is correlation between HER2/neu expressions with lymphnode metastasis in squamous cell carcinoma.

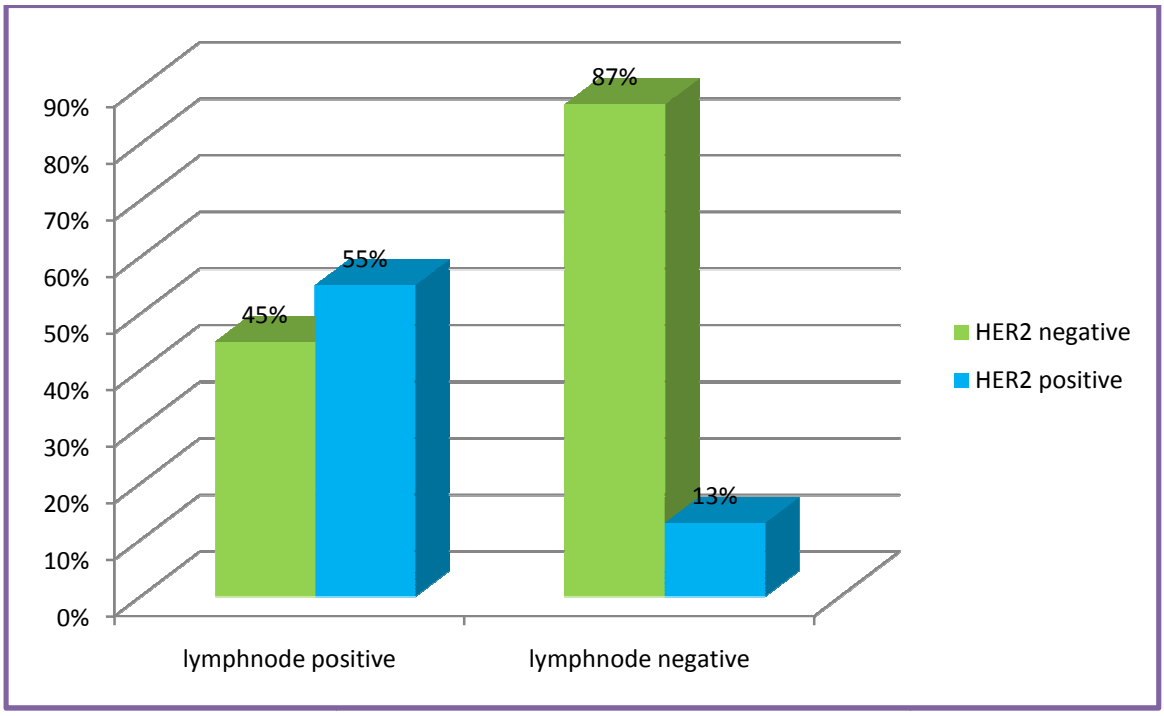

Chart no: 8 Association of her 2 positivity with lymph node metastases in carcinoma cervix

Chart no: 8 shows HER2 /neu expression in lymphnode metastases cases, diagrammatically. Among 9 cases of lymph node metastases, 5cases were positive for HER2/neu (55.55%). Among lymphnode negative cases, there were 12 cases positive for HER2/neu(20%). There was significant correlation between lymphnode metastases and HER2 /neu expression.

PARAMETRIAL EXTENSION:

Parametrial extension is defined as an irregular margin of cervix and soft tissue of the parametrium is more prominent, associated with either a mass of parametrium or loss of periureteral fat plane. The last criteria is a more diagnostic of parametrial extension.

In this study, out of total 100 cases , 30 cases show parametrial extension. Among these 30 cases ,21 cases were of moderately differentiated squamous cell carcinoma, 3 cases were of well differentiated, 3 cases of poorly differentiated carcinoma, 1 case of adenocarcinoma, and 2 cases of small cell carcinoma.

Table 22: Distribution of parametrial involvement in carcinoma cervix:

Type of carcinoma	**Parametrial extension cases(n=30)**
Squamous cell carcinoma	27(90)
Adenocarcinoma	1(3.33%)
Small cell carcinoma	2(6.66)

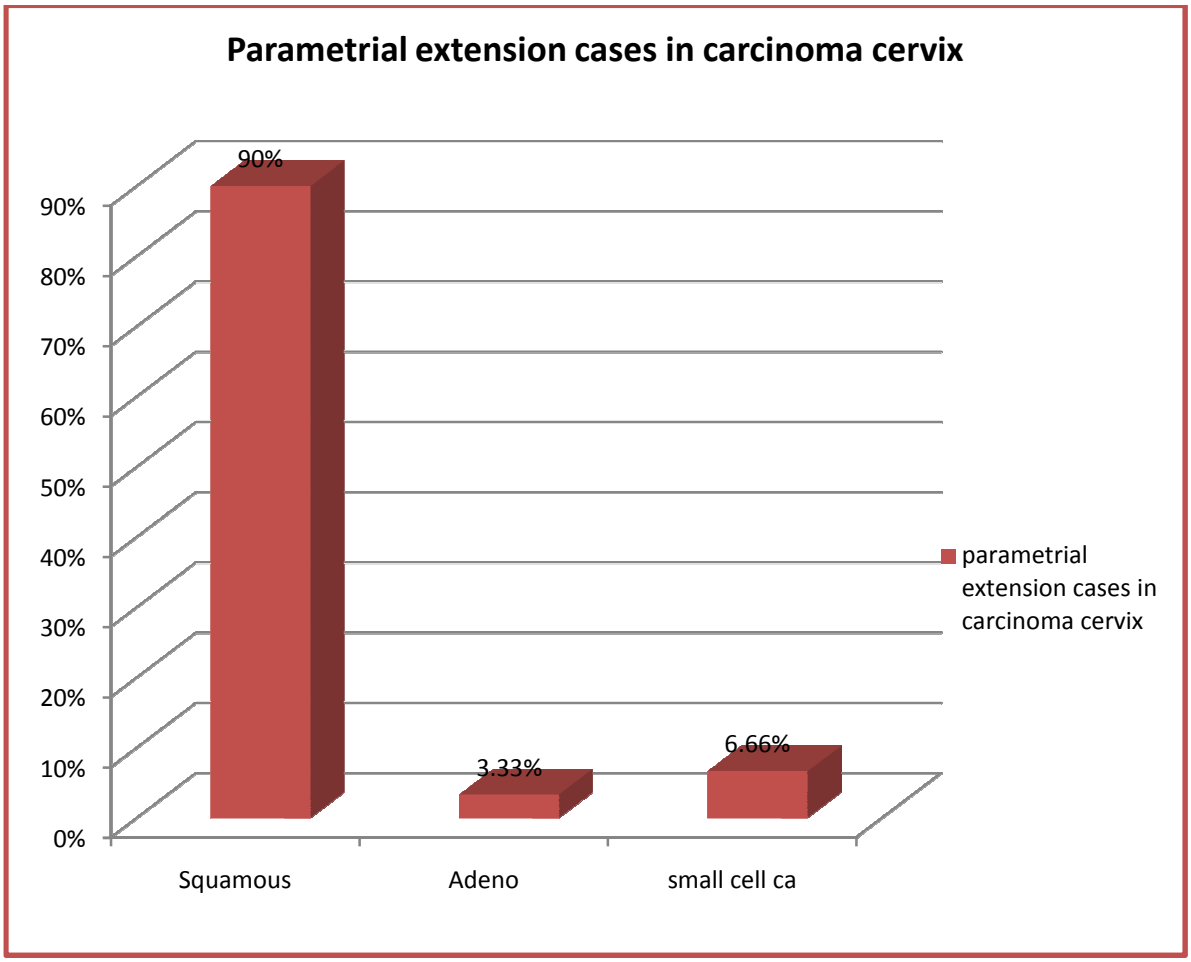

Chart no: 9 Distribution of parametrial extension in carcinoma cervix

Out of 27 cases of squamous cell carcinoma, 21(77.77%) were of moderately differentiated carcinoma, 3(11.11%) were of well differentiated, and 3 (11.11%) were of poorly differentiated carcinoma.

Table 23: Distribution of parametrial extension in grades of squamous cell carcinoma:

Subtype of squamous cell carcinoma	Parametrial extension cases
Well differentiated	3(11.11%)
Moderately differentiated	21(77.7%)
Poorly differentiated	3(11.11%)

Among 30 cases of parametrial extension, 10 cases were positive for HER2 /neu marker. Out of 10 cases,8 cases were of moderately squamous cell carcinoma, 1 was of poorly differentiated, and 1 was of small cell carcinoma.

Table 24: Distribution of HER2/neu positivity in parametrial extension cases of carcinoma cervix:

Type of carcinoma	HER2 Positive cases(n=10)
Squamous cell carcinoma	9(90%)
Small cell carcinoma	1(10%)

Association of HER2/neu positivity with parametrial extension in total 100 cases were made out.

Table 25: Correlation of HER2/neu expression with parametrial extensions in carcinoma cervix:

Parametrial extension	Total no of cases	HER2 positive	HER2 NEGATIVE
Present	30	10(33.33%)	20
Absent	70	7(10%)	63

CHISQUARE TEST=8.103;**P VALUE – 0.0044**

The result is significant .

Table no:25 shows the association between HER2/neu expression and parametrial extension. There were about 30 cases showing parametrial extension. Among this, 10 cases were found positive for HER2/neu (33.33%). There was also significant correlation between parametrial involvement and HER2/neu expression.

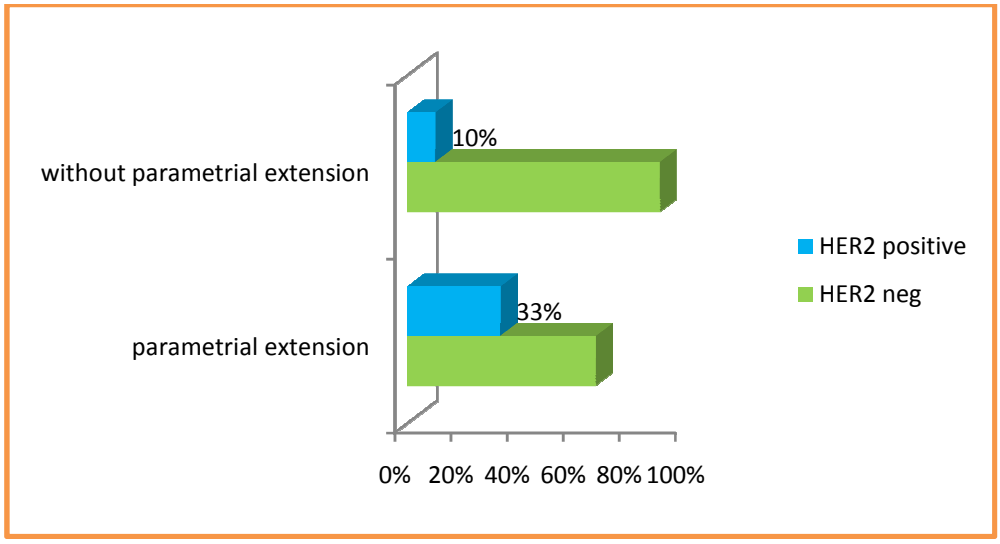

Chart no: 10 Association of HER2 positivity with parametrial extension cases of carcinoma cervix:

The above chart shows HER2/neu expression in parametrial extension cases diagrammatically. Among 30 cases, 10 were positive for HER2/neu oncoprotein (33.33%). As there were 27 cases of squamous cell carcinoma having parametrial extension and out of this 27 cases, 9cases of squamous cell carcinoma had HER2 /neu expression,(90%), their association was studied and correlated.

Table 26: Distribution of HER2/neu expression in parametrial extension cases among grades of squamous cell carcinoma:

Grades of squamous cell carcinoma	**HER2 positive cases in parametrial extension(n=9)**
Well differentiated	Nil
Moderately differentiated	8
Poorly differentiated	1

Table 27: Correlation of HER2/neu with parametrial extension cases of squamous cell carcinoma:

Tumor type- squamous cell carcinoma(n=75)	Present	Absent
Parametrial extension	27(90%)	48
HER2 positivity	9	7

P value is 0.132. the result is not significant. Thus there is no correlation exist between HER2/neu expression with parametrial extension in squamous cell carcinoma.

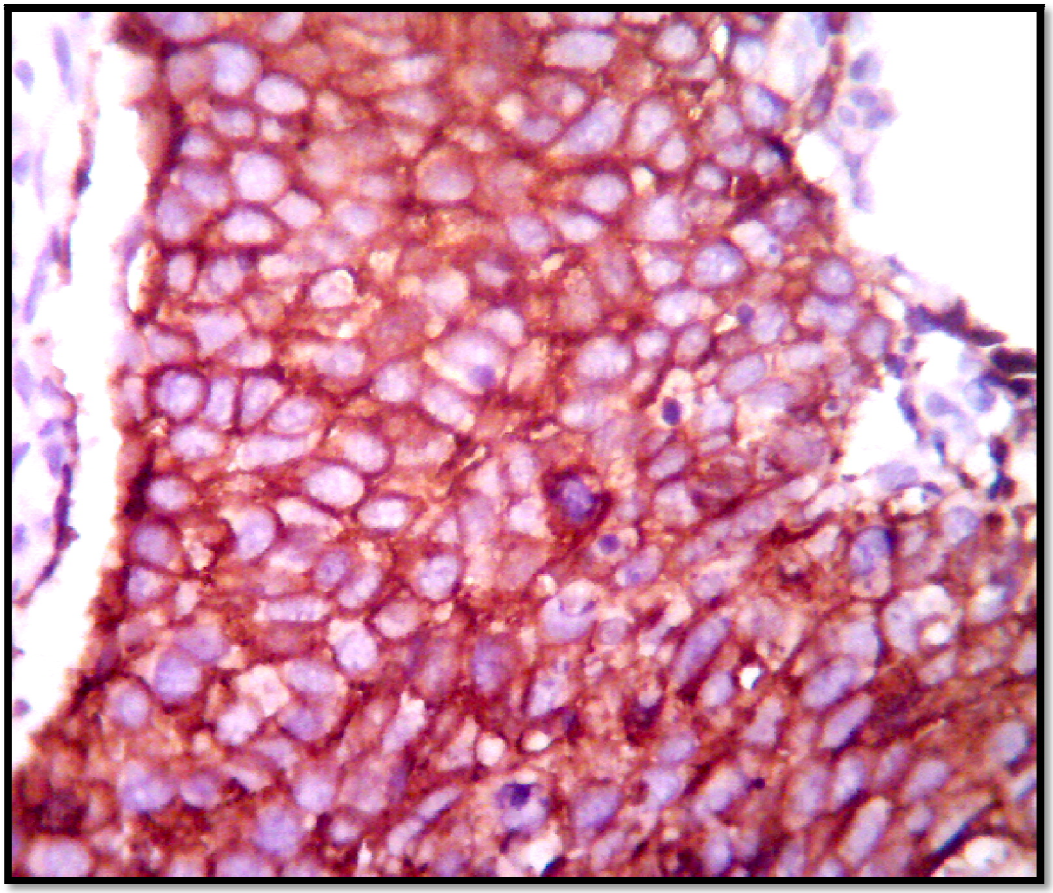

FIG NO:6 - IHC staining of HER2/neu ; score : 3+

Neoplastic squamous cells show strong membranous staining of HER2/neu in 400x(score:3+)

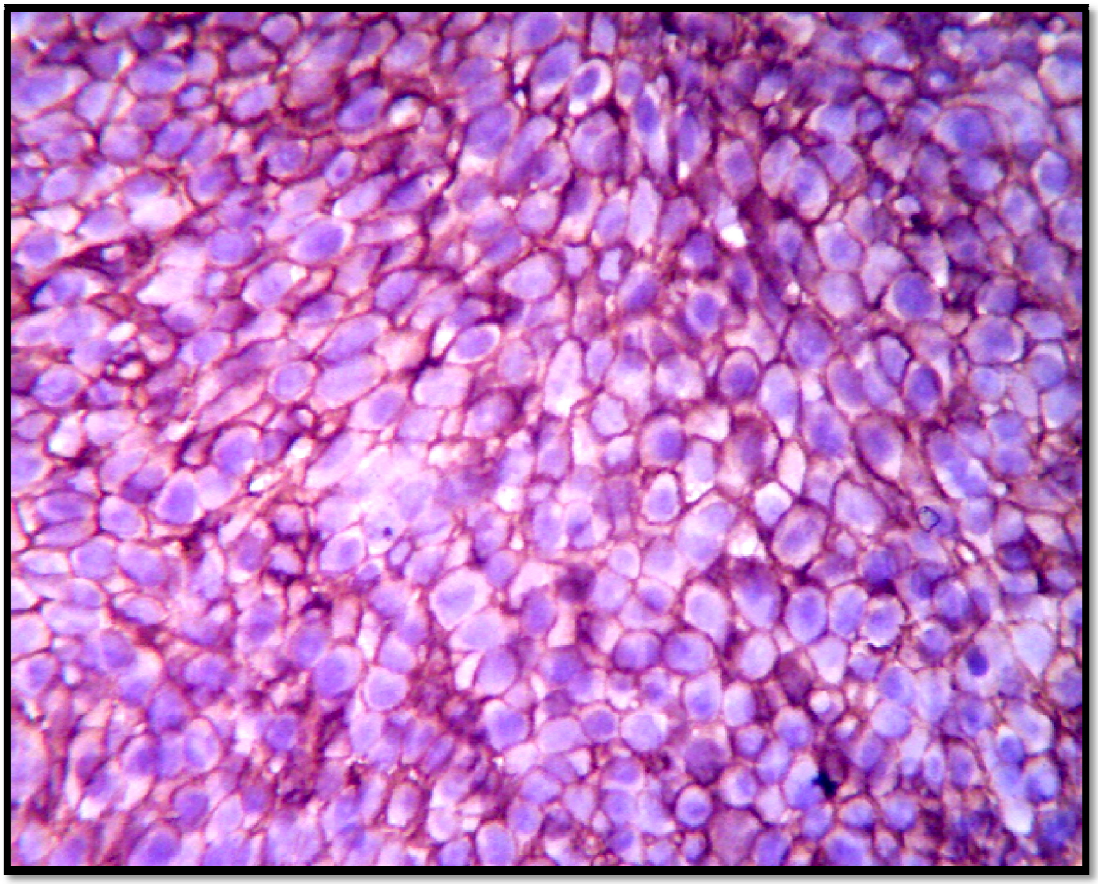

Fig no:7 IHC staining of HER2/neu- score: 3+; 400x

Membranous staining of malignant squamous cells. Score- 3+;400x

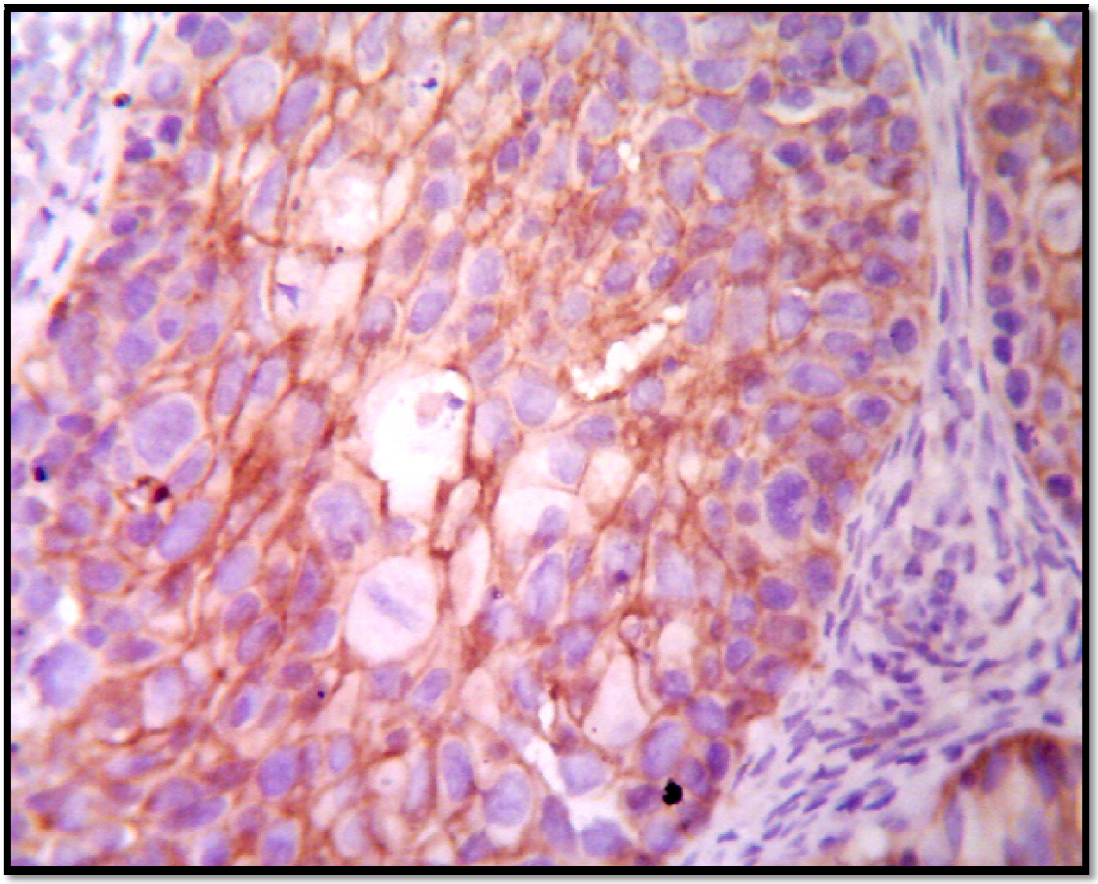

Fig no:8 IHC Staining of HER2/neu – score:2+; 400x

Neoplastic squamous cells show less intense membranous staining .score: 2+, 400x

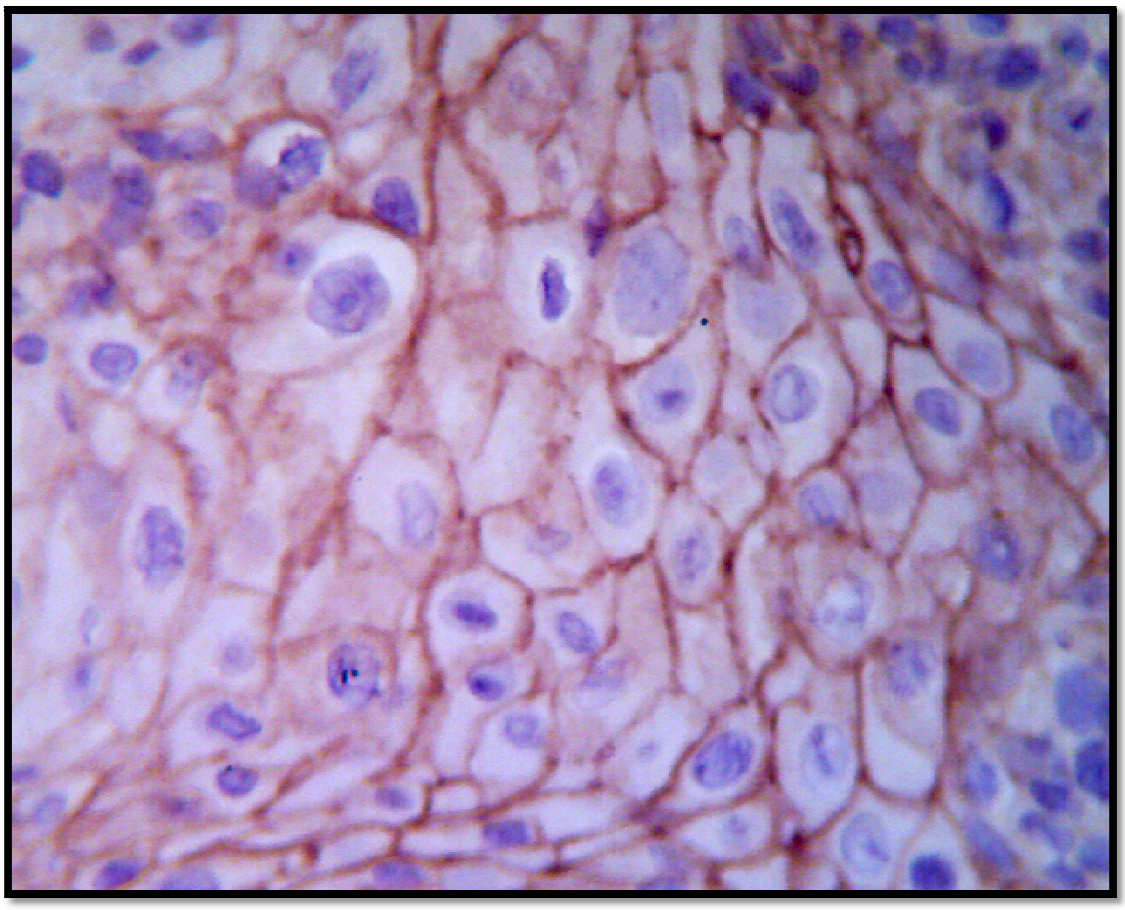

Fig no:9 IHC Staining of HER2/neu : score 2+; 400x

Neoplastic cells with less intense membranous staining, and pleomorphic nuclei, and prominent nucleoli.(HER2 staining- SCORE 2+; 400x)

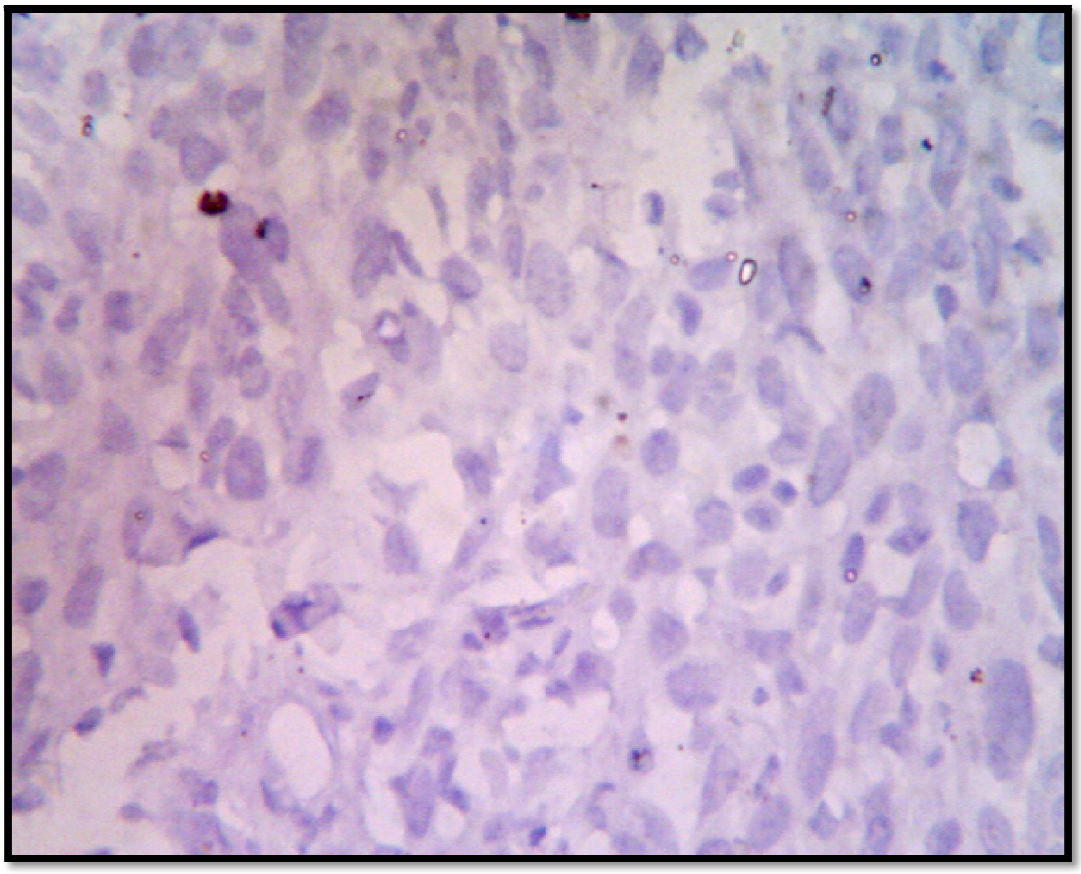

Fig no:11 IHC staining of HER2/neu – score : negative; 400x

This shows negative staining for HER2 in carcinoma cervix ; 400x.

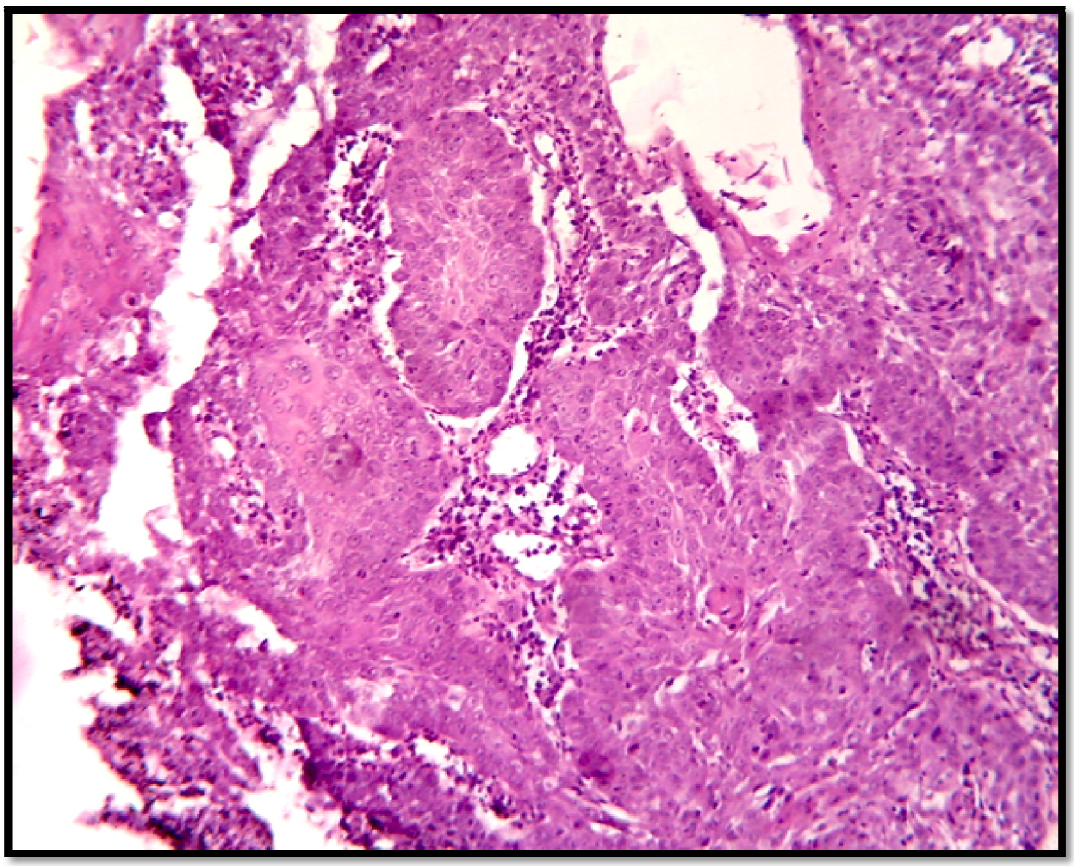

Fig no:12A: H&E stain- Well differentiated squamous cell carcinoma Malignant squamous cell infiltrating the stroma as finger like projections.-well differentiated ;(100x).

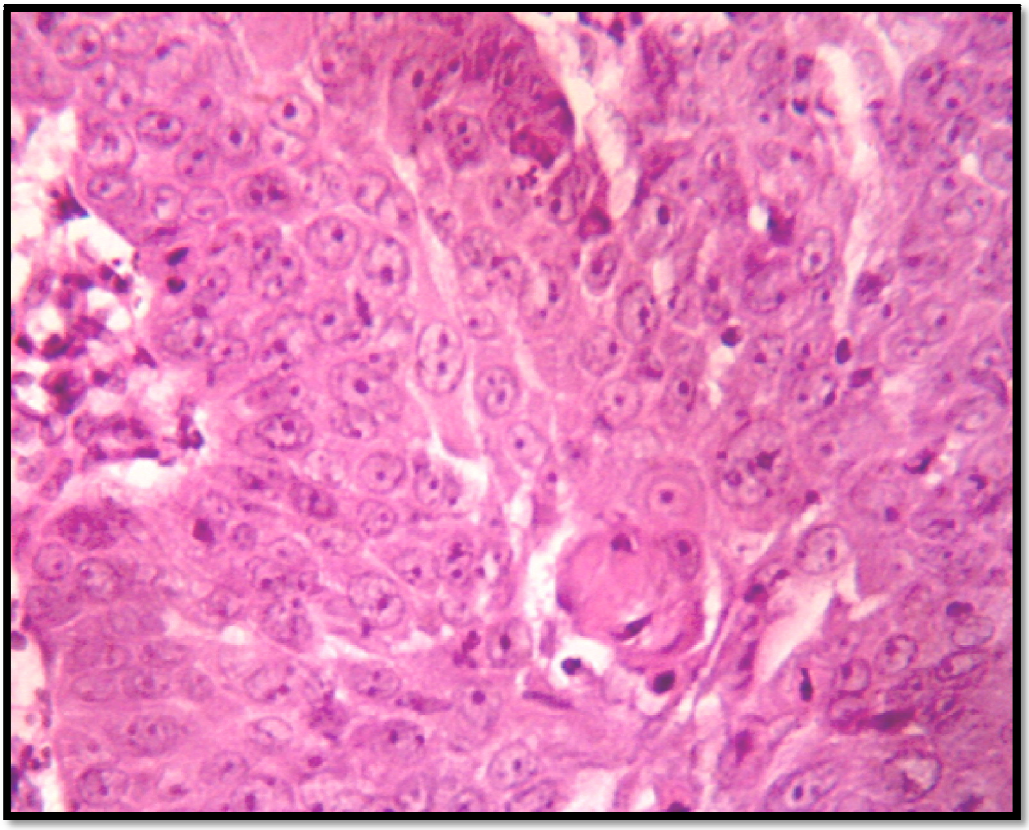

FIG No 12 (B): H&E: Well differentiated squamous cell carcinoma (400x) Cells Are Large, Polygonal , With Pleomorphic Vesicular Nuclei, And Prominent Nucleoli.

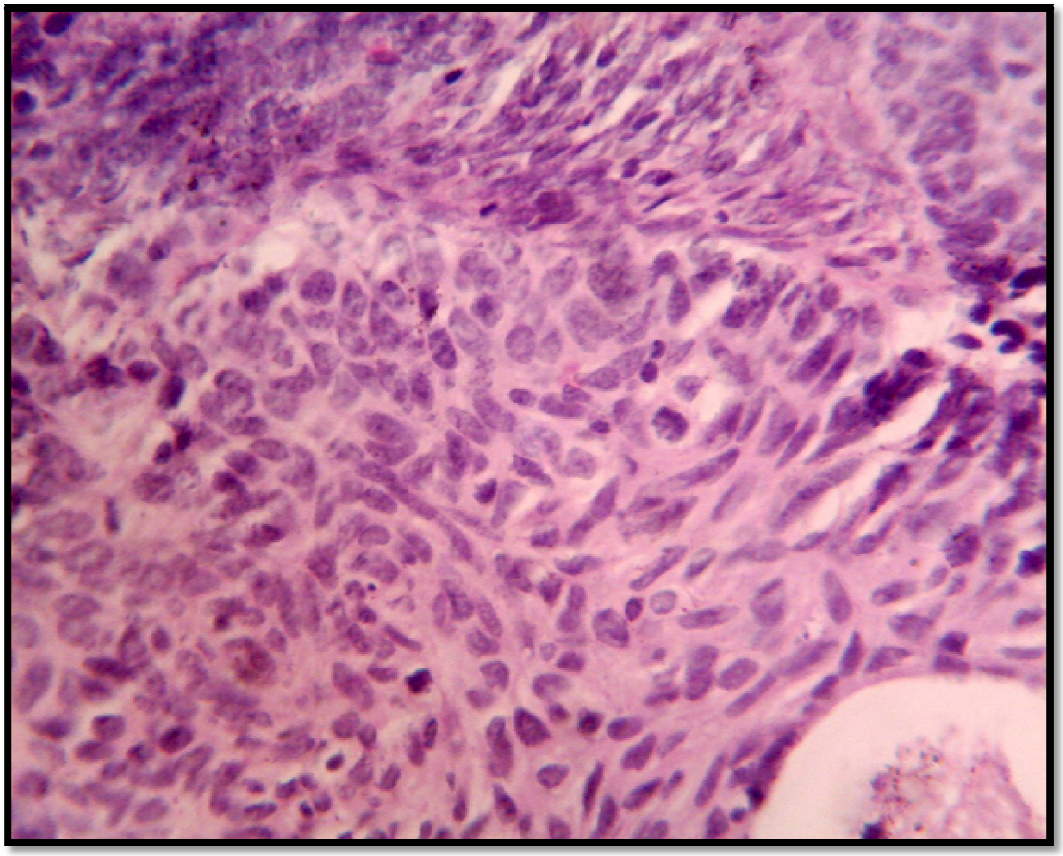

Fig no:18 H& E stain: **Small cell carcinoma**

Malignant squamous cells are small, with nuclear molding – feature of small cell carcinoma.(100x)

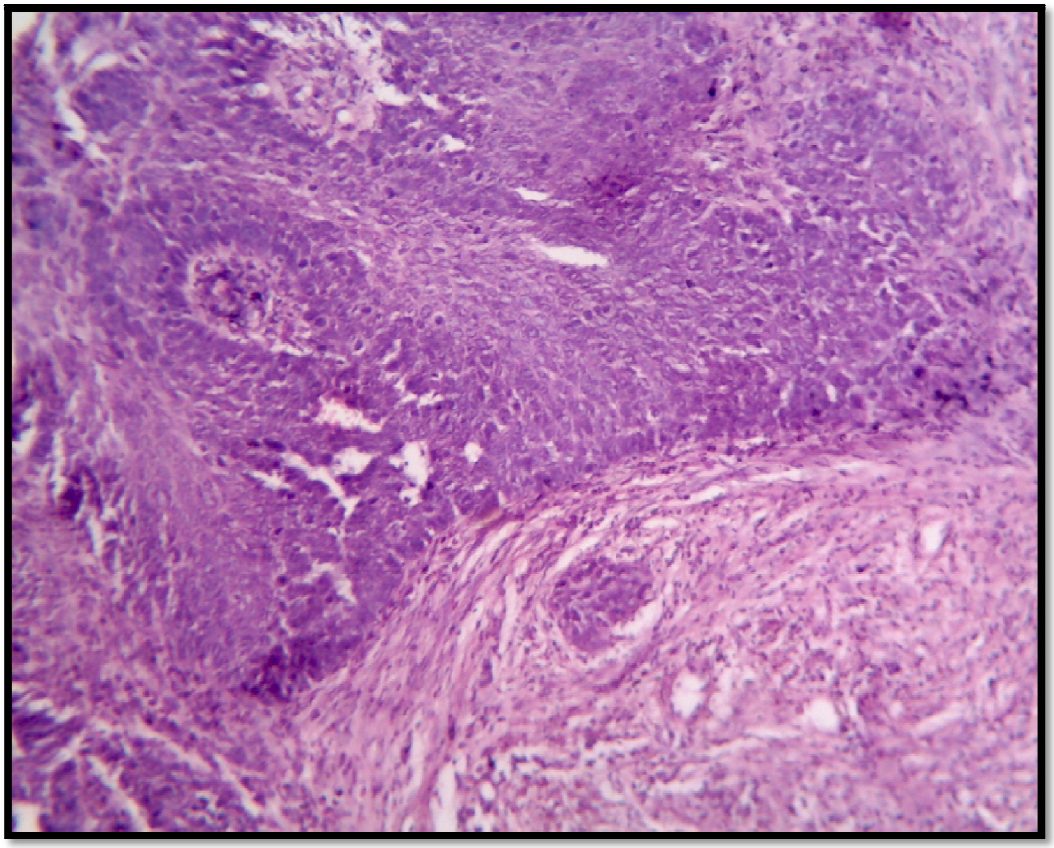

Fig no:15 H&E stain**: Well differentiated carcinoma** : 100x

Squamous cell nest with central necrosis.

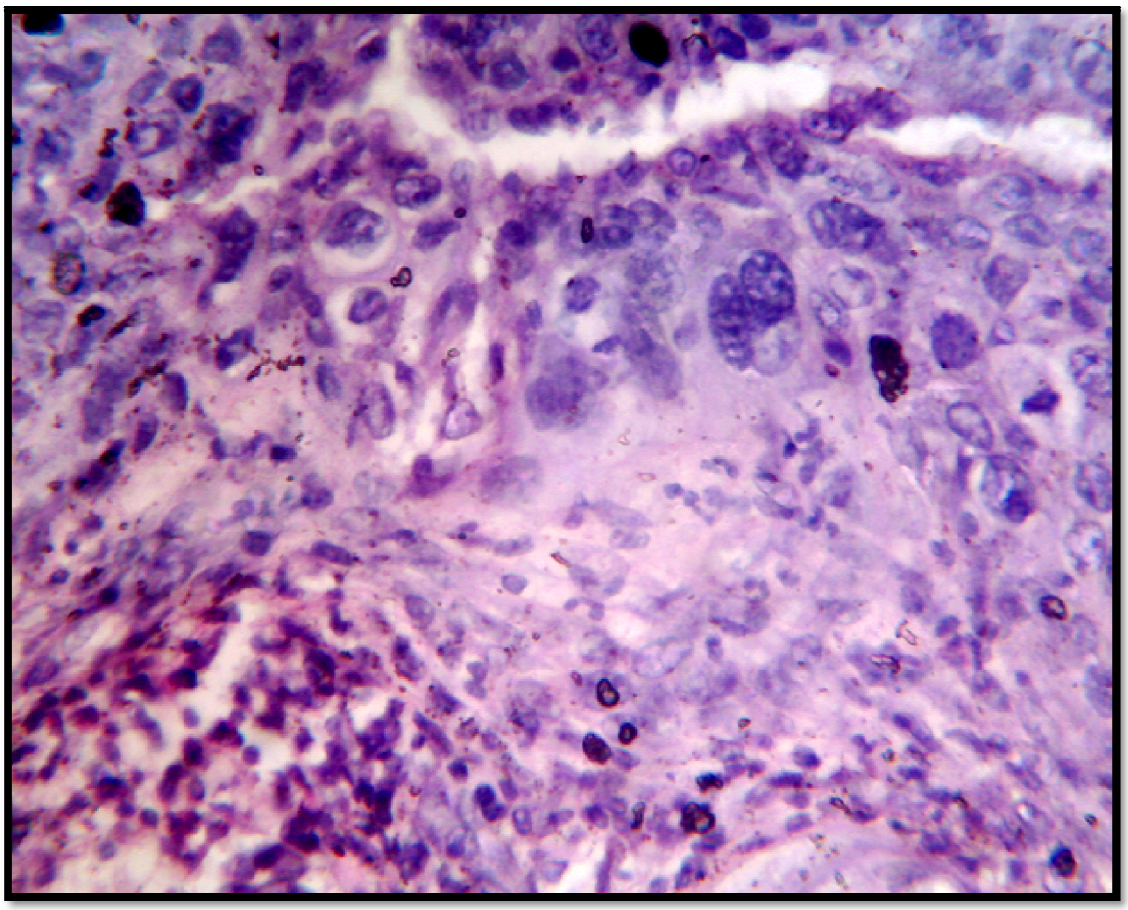

Fig no:17- H&Estain**: Poorly differentiated carcinoma:(400x)**

Individual cells are large, pleomorphic, and mitosis is seen.

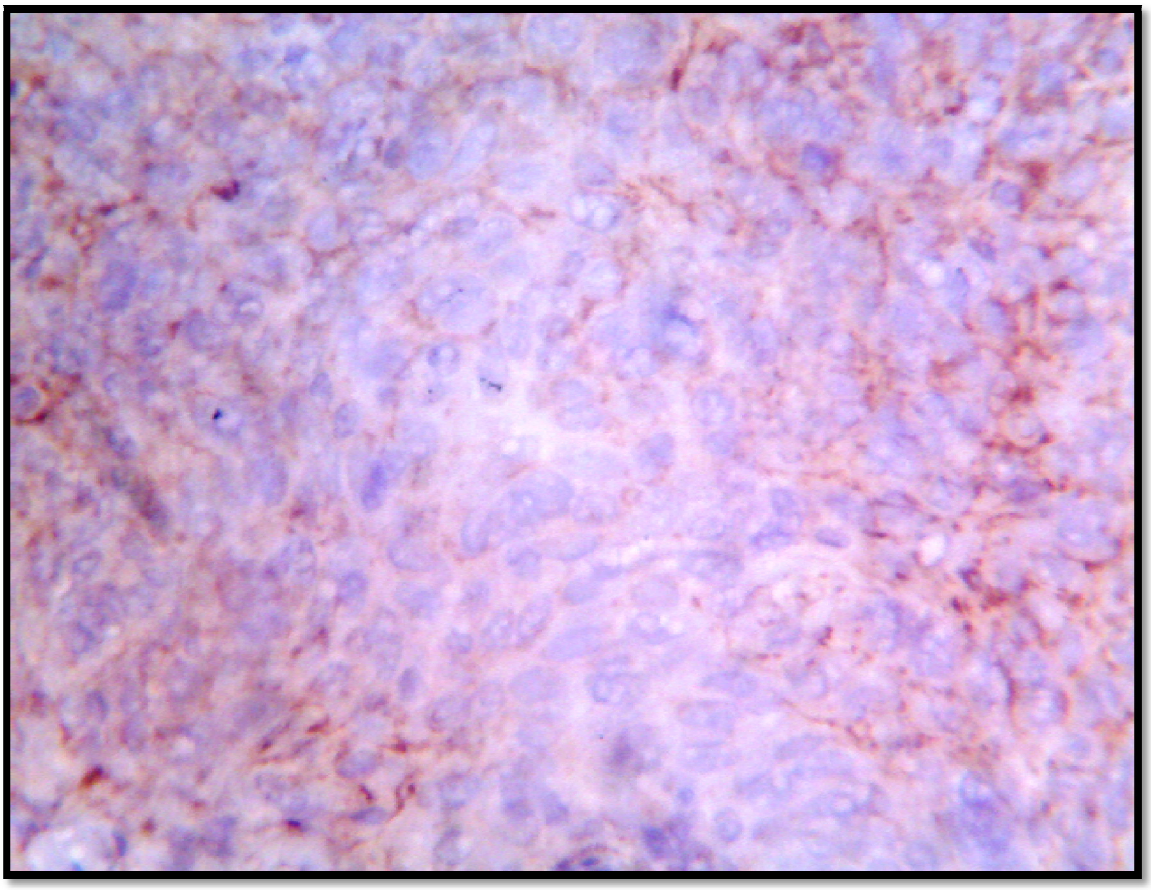

Fig no: 10 IHC staining of HER2/neu – score 1+; 400x

Neoplastic squamous cells with weak membranous staining ; 400x

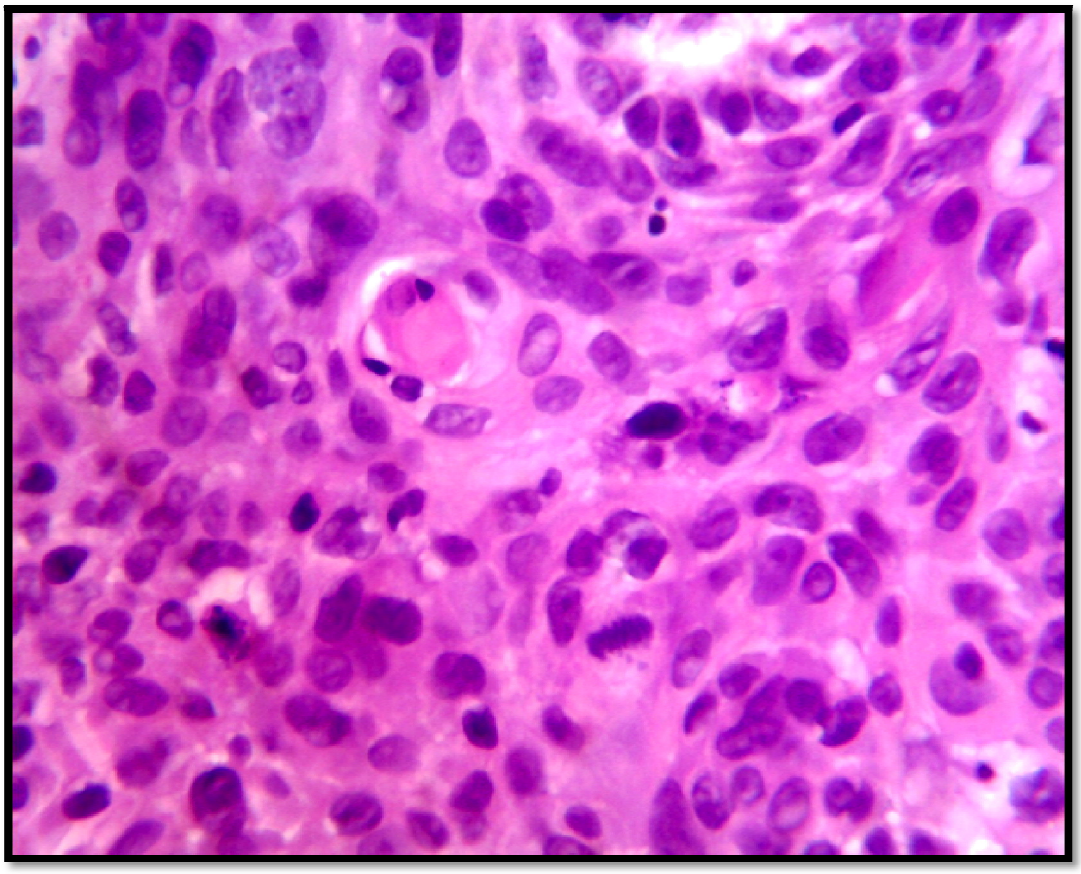

Fig no:16 H&E stain: **Individual cell keratinisation: 400x**

Individual cell keratinisation is seen. Feature of moderately differentiated squamous cell carcinoma.

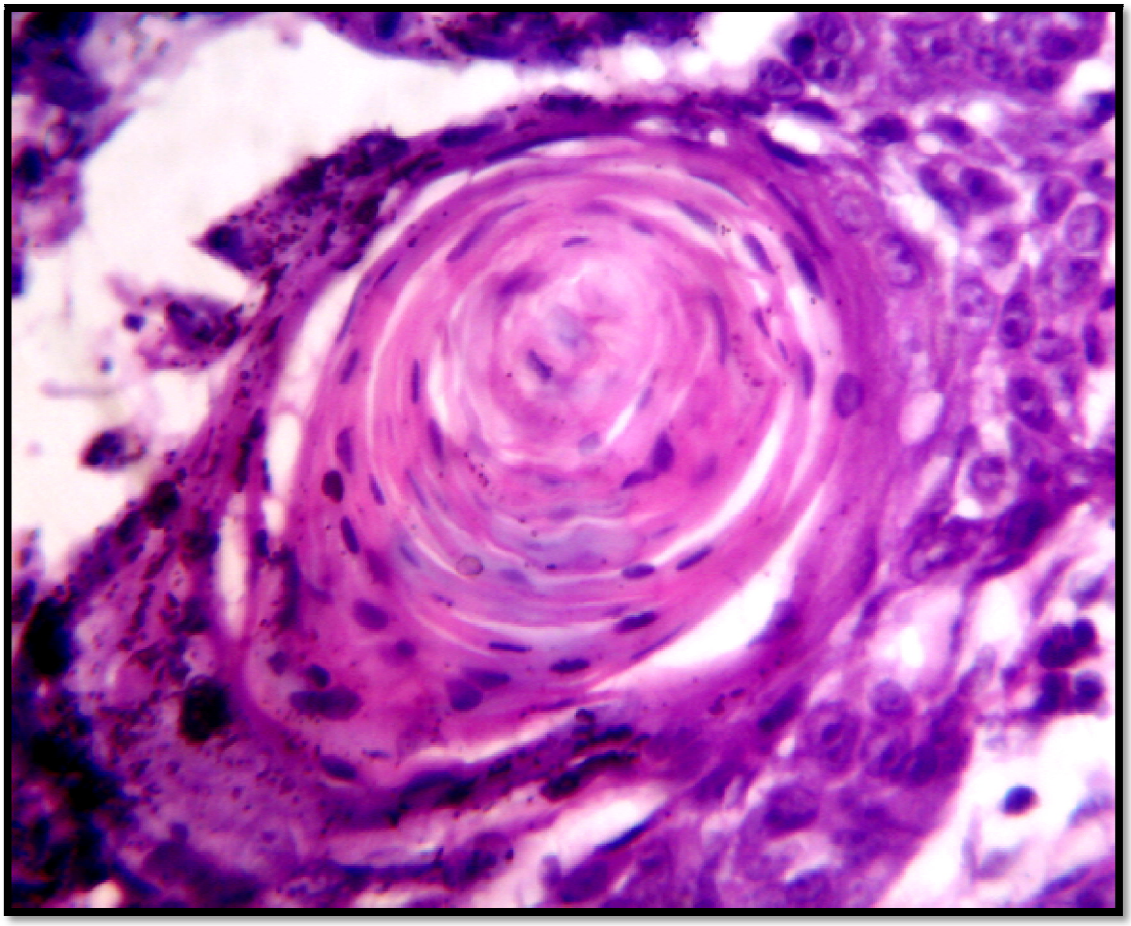

Fig no: 14: H&E **Keratin pearls**

Feature of well differentiated carcinoma ;400x

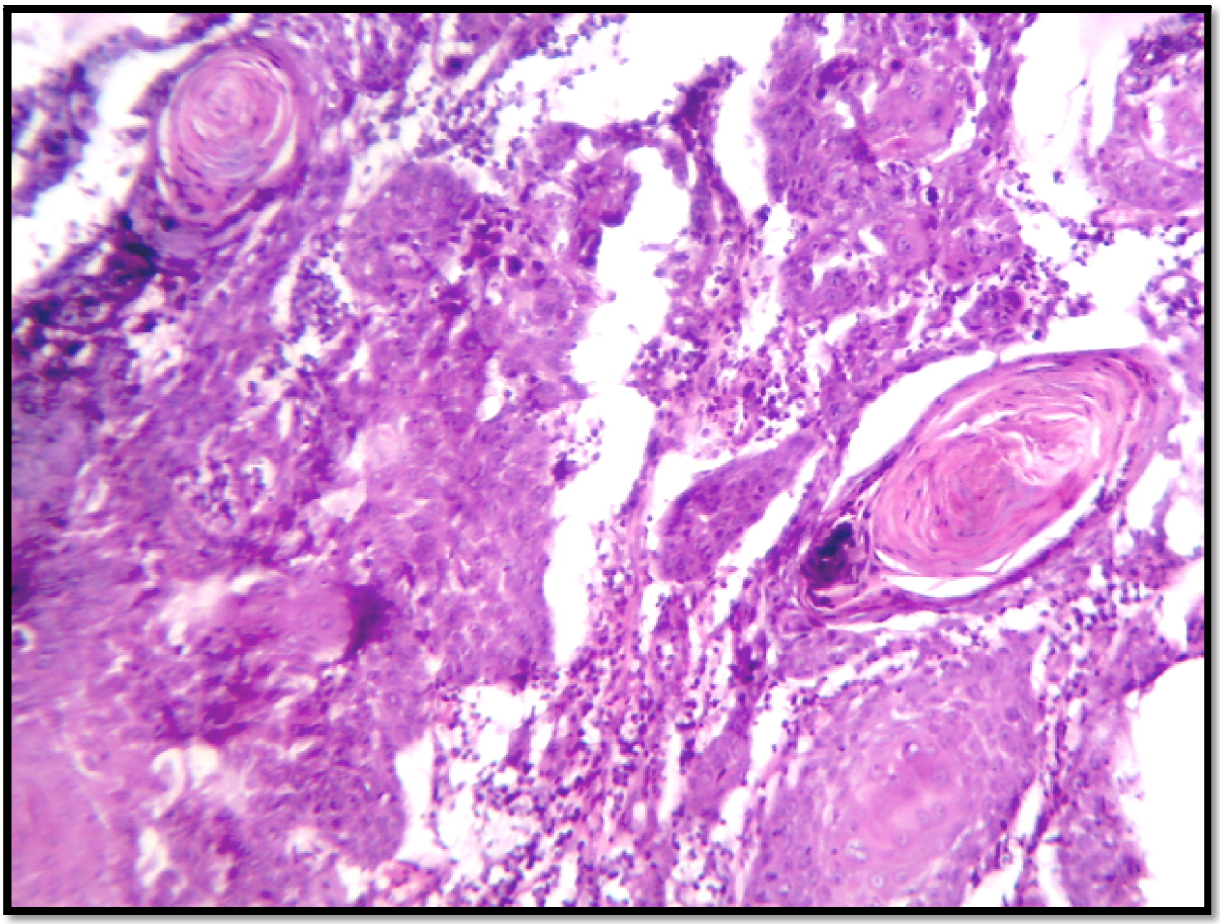

Fig no: 13 H&E stain**: Keratin pearls**

Three keratin pearls in one field.H& E stain(100x)

DISCUSSION

Carcinoma of the uterine cervix remains a major cause of mortality in women in the developing world including India(1). According to recent data provided by the Atlas of Cancer in India project by the ICMR , cervical cancer is the second most common cancer in women after breast cancer in most urban population based registries in India(1), varying from 10.9 to 65.4 amongst various registries with an average incidence of around 25 per 1, 00,000 women(1).

Currently , one of the oncogenes, c-erb-2/HER-2 is thought to be associated with various tumors of the breast, ovary, endometrium, cervix, fallopian tube etc and is being studied extensively. [82] The exact function of HER-2/neu gene product is still unknown but it has tyrosine kinase activity and is thought to function as receptor for growth-regulating molecule.

Activation of HER family members occurs through ligand and a dimer of the same monomer or other member of the HER family that are bound together . Once activation has occurred , tyrosine autophosphorylation of cytoplasmic signal proteins transmit signals to the nucleus , thus regulating aspects of cell growth , division , differentiation and migration. Overexpression of HER2 receptors results in transmitting excessive signals for cell proliferation to nucleus. This may lead to more aggressive growth of the transformed cell. HER2 transformed cells directly contribute to the pathogenesis and clinical aggressiveness of the tumor that overexpress

HER2. The overexpression of HER2 is associated with poor prognosis, including recurrence of the tumor and reduced overall survival.

HER-2/neu over-expression or mutation results in quantitative and qualitative alteration in the membrane proteins which is the basis of its detection. [83],[84] Even though clinical correlation had not been established, many series had suggested that amplification or over-expression of the oncogene might be a marker of poor prognosis in cancers of the ovary, endometrium, breast etc. [85],[86],[87] There were reports in the literature that HER-2/neu over-expression correlates with reduced benefit of adjuvant therapy as in cases of carcinoma breast with tamoxifen therapy. Many studies have shown that regression of HER-2/neu suppresses the malignant phenotypes of cancer cells over-expressing this oncoprotein, which may serve as an excellent target for developing anti-cancer agents specific for HER-2/neu over- expression. [88] HER-2 expression can be estimated either by immunohistochemistry or by fluorescent *in situ* hybridization (FISH). FISH can be used for doubtful cases in IHC to confirm, HER2/neu expression. This combination is not necessary for low (0-1+) or high (3+) grades of immunohistochemical stain as the correlation with gene amplification status is high.

With reviewof the literature available, it was observed that the studies designed to examine HER-2 in lesions of the uterine cervix showed inconsistent results. HER-2 positive staining in invasive carcinoma cervix

ranged from 14-100% in various studies[89,90,91]. Similarly, large variability was also observed in various types of carcinomas, lymph node metastasis and parametrial extension. Several articles on uterine lesions suggest HER-2 positivity is directly related with higher grade and more aggressive tumor, and having poor prognosis.[92] There are also reports revealing that over-expression/amplification of HER-2/neu is uncommon in invasive cervical carcinoma and expression of HER2/neu does not appear to be related to prognosis or treatment outcome.

From the above studies, it was observed that controversy exists regarding the expression of HER-2 in various disorders of the uterine cervix. This study was planned to compare the expression of HER-2 in invasive cervical carcinomas among the women in this region of the country.

In cervical carcinomas, amplification of the *HER-2/neu* gene has been found in 1.5% to 18% of the cases assessed by Southern blot, slot blot, polymerase chain reaction (PCR), or *in situ* hybridization[89]. The rate of expression of HER2/neu assessed by immunohistochemistry has ranged from 9% to 42% [89].

In the carcinoma cervix, correlation between overexpression and clinical stage or survival has been reported in some studies[92] but not in others[93].

Nakano.T et al(1994)[91] studied about prognostic significance of HER2 expression in advanced uterine cervical carcinoma and found that

HER2 positive staining was observed in cancer membrane and cytoplasm and the total positive rate was 43%.

They associated with 5-yr survival rate, and compared with negative groups and found reduced survival in HER2 positive cases and therefore poorer prognosis.

Multivariate analysis using Cox proportional hazards model showed that HER2 was an independent prognostic factor (P = 0.024).

Berchuck A , et al(1990)[90] studied about expression of epidermal growth factor receptor and HER-2/ neu in normal and neoplastic cervix , vulva and vagina. They found that in squamous epithelia of the cervix, vulva, and vagina, epidermal growth factor receptor and HER-2/neu both were expressed most strongly by basal keratinocytes. Expression of both of these cell surface molecules decreased as cells underwent differentiation towards the mucosal surface. In contrast, both epidermal growth factor receptor and HER-2/neu were expressed throughout the entire thickness of the epithelium by undifferentiated squamous cells in squamous metaplasia, raised condyloma and carcinoma in situ. Staining of 34 cases of vulval, vaginal, cervical lesions, 33 of these cancers for HER-2/neu was weak, although one patient who presented with distant metastases had strong staining for HER-2/neu.

Fadare, Oluwole M.d ., et al(2004)[94] found that immunohistochemical study of HER2 expression in tissue microarray containing 169 cases of

cervical carcinoma comprised of cancer staging Ia and Ib cases. Among this squamous cell carcinoma constitute about 110 cases, 36 were adenocarcinoma, 13 were adenosquamous carcinoma, neuroendocrine tumor were about 2 in number, adenocarcinoma with neuroendocrine differentiation were 2 in number, mixed squamous cell carcinoma was 1 in number.HER2neu expression was <1%. Rosy et al have noted , strikingly discrepant results regarding the frequency of HER2/neu overexpression ranging from <1% to 42%. Both of them , had increased HER2neu expression in small cell carcinoma.

Nakano T et al (1994), found c-erbB-OncoProtein Expression(Cerb B-OPE) was observed on the cell membrane of carcinoma cells. Positivity of CerbB-OPE, which showed differences among histologic subtypes , in early cervical squamous cell carcinoma.

Nidhi gupta et al ,(2009)[95] observed in their study that 63% cases of carcinoma cervix showed definite membranous staining for c-erbB-2 oncoprotein and these included squamous cell carcinoma (54.1%), adenocarcinoma (84.61%), adenosquamous carcinoma (100%) and CIN (60%). It was also observed that the positivity varied with differentiation and staging, lymph node metastasis and parametrial extension status. Poorly differentiated squamous cell carcinoma showed 80% positivity whereas positivity was 87.5% and 100% in case of Stage-III and IV tumors . Cases with lymph node metastasis revealed 92.86% positivity whereas with

parametrial extension revealed 71.88% positivity. Out of 14 cases of lymph node metastasis, squamous cell carcinoma (11 cases) and adenocarcinoma (three cases) showed c-erbB-2 positivity rate of 90.9% and 100% respectively. There were 32 cases of parametrial extension of which HER-2 was positive in squamous cell carcinoma (67.86%), adenocarcinoma (100%), adenosquamous carcinoma (100%) .

The present study included 100 cases of cervical biopsies and Abdominal hysterectomies which were staged by FIGO staging from stage I b to IIIb. The histological subtypes comprised of 75 cases of squamous cell carcinomas, 21 cases of adenocarcinomas,3 cases of small cell carcinoma, and 1 case of adenosquamous carcinoma . Among these cases, 17cases (17%) were found to be positive for HER2/neu expression.

COMPARISON OF PERCENTAGE OF HER2/neu EXPRESSION IN PRESENT STUDY WITH OTHER STUDIES: (Table 28)

Comparing the HER2positivity with other studies, it were seen that about 43% of cases showed positive for HER2 in Nakano.et al study[91], <1% in Fadare.,et al study[94],and 63% in Gupta .et al study[95]. Among these studies, present study have more similarities with Gupta.,et al study.

TABLEno:28 Comparision of % of HER2 neu expression among different studies

Name of study	HER2 expression
Nakano .et al (1994) [91]	43%
Fadre ., et al (2004) [94]	<1%
Gupta. et al (2009)[95]	63%
Present study	17%

COMPARISON OF HER2 EXPRESSION IN HISTOLOGICAL TYPES OF CARCINOMA CERVIX : (Table 29)

Among 17 cases, 16 cases of squamous cell carcinoma (21.33%) , and 1 case (33%) of small cell carcinoma were positive for HER2 oncoprotein. The positivity rate varied with different hitological types, and with varying stages, presence of lymph node metastases, and parametrial extension.

Gupta. Et al got 54.1% positivity in squamous carcinoma ,whereas present study showed 21.33% only, in squamous carcinoma. It was 84.61% positivity in Adenocarcinoma and was 100% in Adenosquamous carcinoma in the study of Gupta .et al. But the present study got no positivity in both adenocarcinoma as well as in adenosquamous carcinoma.

TABLE no 29: Comparison of HER2 expression in histological types of carcinoma cervix between Gupta. Et al study and this study:

Name of study	Squamous cell carcinoma	Adenocarcinoma	Adenosquamous carcinoma	Small cell carcinoma
Gupta. Et al (2009)[95]	54.1%	84.61%	100	Nil
This study	21.33%	Nil	Nil	33.33%

In squamous cell carcinoma, high positivity rate(25%) was for poorly differentiated carcinoma and among other types of carcinoma, small cell carcinoma had high positivity rate(33.33%).

COMPARISON OF HER2/neu EXPRESSION IN GRADES OF SQUAMOUS CELL CARCINOMA:(Table 30)

In the present study , HER 2/neu positivity in well differentiated carcinoma is 13.04%, moderately differentiated carcinoma is 25%, and poorly differentiated carcinoma is 25%. In Gupta.et al study, the HER2/neu positivity in moderately differentiated carcinoma is 54.55%, and in poorly differentiated carcinoma is 80%.

Table 30: Comparison of HER2/neu expression in grades of Squamous cell Carcinoma of cervix between this study and Gupta.et al study:

Name of study	Well differentiated carcinoma	Moderately differentiated carcinoma	Poorly differentiated carcinoma
Gupta.et al(2009)[95]	0	54.55%	80%
This study	13.04%	25%	25%

The present study when compared with Gupta.et al study, it is seen that well differentiated carcinoma showed 13.04%, whereas it is negative in Gupta.et al study. There is increased expression of HER2/neu with higher grade. But in the present study , the expression is of same percentage (25%) in both moderately and poorly differentiated carcinoma .

COMPARISON OF HER2/neu EXPRESSION IN CLINICAL STAGE OF CARCINOMA CERVX: (Table 31)

There was increased positivity rate in stage III(50%) of cervical carcinoma, followed by stage II (18%), stage I (9%). In the present study, stage IV carcinoma was not included as the block was not available . In Gupta .et al study, highest HER2 positivity was for stage IV carcinoma (100%), followed by stage III (87.5%), stage II (72%), and stage I (48%).

TABLE no:31 Comparison of distribution HER2 expression in clinical stages of carcinoma cervix in Gupta. Et al study this study:

Name of study	Stage I	Stage II	Stage III	Stage IV
Gupta .et al (2009)[95]	48%	72%	87.5%	100%
This study	9.37%	18%	50%	Nil

In both study , there is increasing order of HER2 expression as the stage of the cancer progresses , but in varying rates.

The present study shows significant co-relation(p value- 0.0016) exists between HER2 expression and stage of the tumor. Gupta .et al study also had significant p value(<0.05).

COMPARISON OF HER2/neu EXPRESSION IN LYMPH NODE METASTATIC CASES OF CARCINOMA CERVIX: (Table 32)

Lymphnode metastases were found in 9 cases which belongs to squamous cell carcinoma. Among this 9 cases, 5 cases(55.55%) were positive for HER2 protein. In Gupta .et al study , lymphnode metastasis were seen in 14 cases and among this ,13 cases were positive for HER2(92.86%).

TABLE no 32 :Comparison of HER2 expression in lymphnode metastatic case of carcinoma cervix in Gupta . et al and present study.

Name of study	Total cases of lymphnode metastases	HER2 positivity
Gupta .et al (2009)[95] study	14	13(92.86%)
Present study	9	5(55.55%)

Thus in both study , there were increased expression of HER2 in lymphnode metastasis cases.

COMPARISON OF HER2/neu EXPRESSION IN PARAMETRIAL EXTENSION CASES OF CARCINOMA CERVIX: (Table 33)

There were 30 cases of parametrial extension ,which comprises of 27 cases of squamous cell carcinomas, 2 cases were of small cell carcinoma, 1case of adenocarcinoma. Among this ,10 cases(33.33%) show increased expression of HER2 oncoprotein. Gupta .et al group had total of 32 cases of parametrial extension and among this 32 cases , 23 cases (71.88%)were positive for HER2/neu.

TABLE no 33:Comparison of HER2 expression in parametrial extension of carcinoma cervix in Gupta .et al and present study:

Name of study	Total cases	HER2 positivity
Gupta.et al (2009)[95]	32	23(71.88%)
Present study	30	10(33.33%)

The both studies show increased HER2expression in parametrial extension cases. Compared to HER2 expression in lymphnode positive cases and parametrial extension, lymphnode positive cases showed increased positivity.

There was a significant correlation between positive HER2 expression and higher clinical stage of presentation .HER2 expression was significantly higher in lymph node positive cases than in lymphnode negative cases .There was also significantly high expression in cases with parametrial involvement. In other study by Nidhi Gupta et al, there was positivity in adeno carcinoma and adenosquamous carcinoma. But in present study , there was no positivity in adeno and adenosquamous carcinoma. In contrast, there was positivity in small cell carcinoma of about 33.33 % . Other than histological types, results correlate well with other similar studies.

In the literature , there are reports in favour of increased expression of HER2 oncoprotein and also reports saying that there is only rare overexpression of HER2 oncoprotein.

Thus , there are contradictory reports on expression of HER-2/neu and prognosis in various uterine lesions which may either be due to heterogeneity of lesions or technical problem with antigen retrieval. HER-2/neu has a complex activation pathway and its expression is controlled not only by the degree of gene amplification but also by several other factors like gene receptor alteration and rate of gene transcription, which help in tyrosine kinase activation leading to cellular transformation. [96].Steroid hormones can also modulate gene expression by direct binding of hormone receptor complexes to specific DNA regulating sites. [97],[98] It is quite evident that modification in any of these factors can alter the over-expression of HER-2/neu, thus altering the positivity rates on immunostaining in various malignant neoplasms including carcinoma cervix. Whether or not these factors are associated with the prognosis, is a matter of further investigation.

SUMMARY

1. HER2/neu expression in invasive cervical carcinoma is 17%
2. For higher clinical stage of carcinoma cervix, HER2/neu expression is more (stage III carcinoma-50%).
3. HER2/neu expression is more in carcinoma cervix cases with lymphnode metstatic cases(55.5%) and cases with parametrial extension cases(33.33%).
4. HER2/neu expression is more in moderately differentiated carcinoma. poorly differentiated carcinoma of cervix(25%) and Small cell carcinoma of cervix (33.33%).

CONCLUSION

1. This study shows HER2/neu expression in cervical carcinoma correlates with higher clinical stage (stage III).
2. HER 2 /neu expression correlates with Histological types of carcinoma Cervix.
3. HER2/neu expression in carcinoma cervix correlates with lymphnode metastasis and with parametrial extension .
4. Expression of HER2/neu in carcinoma cervix, in this study is lower , when compared to other similar studies. Hence further studies on various factors associated with HER2 expression and its positivity rates in various subtypes and their prognostic significance are needed.

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ANNEXURE-I

PROFORMA

Case number :

Name :

Age :

Sex :

HPE number :

IP number :

Clinical history :

Risk factors, if any :

Clinical diagnosis :

Imaging :

Previous HPE report :

Nature of specimen : cervix biopsies, or total abdominal hysterectomies

GROSS :

Size of the tumor in hysterectomy specimen, total nodes dissected, parametrial extension

MICROSCOPY

Histological type :

Histological grade : well differentiated, moderately differentiated, and poorly differentiated carcinoma of squamous cell carcinoma

Associated findings :

Total number of nodes dissected: Number of nodes involved:

Parametrial extension, Distant metastasis

FIGO staging :

IMMUNOHISTOCHEMISTRY

HER2/neu score: 1+,2+,3+, negative

ANNEXURE -II

WHO CLASSIFICATION

EPITHELIAL TUMORS:

Squamous tumors and precursors

Squamous cell carcinoma, not otherwise specified

- Keratinising
- Non keratinising
- Basaloid
- Verrucous
- Warty
- Papillary
- Lymphoepithelioma
- Squamotransistional

Early invasive (microinvasive) squamous cell carcinoma

Squamous intraepithelial neoplasia

- Cervical intraepithelial neoplasia(CIN3)
- Squamous cell carcinoma in situ

Benign squamous cell lesions

- Condyloma accuminatum
- Squamous papilloma
- Fibroepithelial polyp

Glandular tumors and precursors

Adenocarcinoma

Mucinous adenocarcinoma

- Endocervical
- Intestinal
- Signet-ring cell
- Minimal deviation
- Villoglandular
- Endometrioid adenocarcinoma
- Clear cell adenocarcinoma
- Serous adenocarcinoma
- Mesonephric adenocarcinoma

Early invasive adenocarcinoma

Adenocarcinoma in situ

Glandular dysplasia

Benign glandular lesions

- Mullerian papilloma
- Endocervical polyp

Other epithelial tumors

Adenosquamous carcinoma

- Glassy cell carcinoma variant

Adenoid cystic carcinoma

Adenoid basal carcinoma

Neuroendocrine tumors

- Carcnoid
- Atypical carcinoid
- Small cell carcinoma
- Large cell neuroendocrine carcinoma

Undifferentiated carcinoma

MESENCHYMAL TUMORS AND TUMOR LIKE CONDITIONS

Leiomyosarcoma

Endometrioid stromal sarcoma, low grade

Undifferentiated endocervical sarcoma

Sarcoma botryoides

Alveolar soft part sarcoma

Angiosarcoma

Malignant peripheral nerve sheath tumor

Leiomyoma

Genital rhabdomyoma

Postperative spindle cell nodule

MIXED EPITHELIAL AND MESENHYMAL LESIONS

- Carcinomasarcoma
- Adenosarcoma
- Wilms tumor
- Adenofibroma
- Adenomyoma

Melanocytic tumors

MISCELLANEOUS TUMORS

Tumors of germ cell type

- Yolk sac tumor
- Dermoid cyst
- Mature cystic teratoma

Lymphoid hematopoeitic tumors

Secondary tumors

ANNEXURE III

Staging is done by FIGO staging(94)

Stage I	Cervical carcinoma confined to uterus (extension to the corpus should be disregarded)
Stage IA	Invasive carcinoma diagnosed only by microscopy; all macroscopically visible lesions, even with superficial invasion, are stage IB
Stage IA1	Stromal invasion no greater than 3.0 mm in depth and 7.0 mm or less in horizontal spread
Stage IA2	Stromal invasion more than 3.0 mm and not more than 5.0 mm with a horizontal spread of 7.0 mm or less
Stage IB	Clinically visible lesion confined to the cervix or microscopic lesion greater than IA2a
Stage IB1	Clinically visible lesion 4.0 cm or less in greatest dimension
Stage IB2	Clinically visible lesion more than 4.0 cm in greatest dimension
Stage II	Tumor invades beyond the uterus but not to pelvic wall or to lower third of the vagina
Stage IIA	Without parametrial invasion

Stage IIA1	Clinically visible lesion _4.0 cm in greatestdimension
Stage IIA2	Clinically visible lesion >4 cm in greatest dimension
Stage IIB	With parametrial invasion
Stage III	Tumor extends to the pelvic wall and/or involves lower third of vagina and/or causes hydronephrosis or nonfunctioning kidney
Stage IIIA	Tumor involves lower third of vagina with no extension to pelvic wall
Stage IIIB	Tumor extends to pelvic wall and/or causes hydronephrosis or nonfunctioning kidney
Stage IV	The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema, as such, does not permit a case to be allotted to stage IV
Stage IVA	Spread of the growth to adjacent organs
Stage IVB	Spread to distant organs

ANNEXURE IV

HAEMATOXYLIN & EOSIN STAINING TECHNIQUE[90]

PREPARATION OF HAEMATOXYLIN SOLUTION:

Haematoxylin	2. 5gm
Mercuric oxide	1.25gm
Potassium alum	50gm
Absolute ethyl alcohol	25ml
Sodium iodate	0.5gm
Distilled water	500ml

PROCEDURE:

Potassium alum, 50gm is dissolved in 500ml of distilled water by heating and shaking at 60^0 C. Add solution of 2.5gm of Haematoxylin in 25ml of absolute ethyl alcohol and bring rapidly to boil. When it begins to boil, remove from flame and add 1.25gm of mercuric oxide or sodium iodate. Mix by swirling gently.

PREPARATION OF EOSIN SOLUTION:

Eosin Y	1gm
95% Ethanol	80ml
Glacial Acetic acid	0.2ml
Distilled water	20ml

PROCEDURE:

Dissolve 1gm Eosin Y in 20ml of distilled water and add 80ml of 95% etanol and 0.2ml of glacial acetic acid.

STAINING PROCEDURE:

1.Xylene 3 changes-2mins each.

2.90%, 80%, 70% alcohol-10 dips each.

3. Bring sections to water.

4. Harris Haematoxylin-15 minutes.

5. Rinse in tap water.

6. Differentiate in 1% acid alcohol.

7. Rinse in tap water.

8. Lithium carbonate 0.5%- until blue.

9. Tap water wash.

10. Eosin-15secs to 2mins depending on age of Eosin.

11. Rinse in tap water.

12. Dehydrate in 95% alcohol.

13. Absolute alcohol-3 changes-10 dips each.

14. Xylene 2 changes-10 dips.

15. Mount in DPX mountant.

RESULTS:

Nuclei- Blue

Cytoplasm, RBCs, Keratin- Pink

Eosinophil granules- Orange red

ANNEXURE V

KEY FOR MASTER CHART:

1 - Squamous cell carcinoma, well differentiated

2 - Squamous cell carcinoma, moderately differentiated

3 - Squamous cell carcinoma, poorly differentiated

4- - Small cell carcinoma

5 - Adenocarcinoma

6 - Adenosquamous carcinoma

Sl.No	IP No.	AGE	SEX	H.P.NO	TYPE OF SPECIMEN	CLINICAL STAGING	HISTOLOGICAL DIAGNOSIS	HER2 STATUS	LYMPHNODE INVOLVEMENT	PARAMETRIAL EXTENSION
1	12594/13	50	F	416/13	BIOPSY-CERVIX	IB	2	2+	ABSENT	ABSENT
2	13315/13	49	F	430/13	BIOPSY-CERVIX	IIIB	2	NEGATIVE	PRESENT	PRESENT
3	13581/13	68	F	436/13	BIOPSY-CERVIX	II B	2	NEGATIVE	ABSENT	PRESENT
4	14041/13	50	F	485/13	BIOPSY-CERVIX	IB	1	NEGATIVE	ABSENT	ABSENT
5	15253/13	48	F	503/13	HYSTERECTOMY	IIIB	2	NEGATIVE	PRESENT	PRESENT
6	16556/13	70	F	600/13	BIOPSY-CERVIX	IB	2	NEGATIVE	ABSENT	ABSENT
7	18277/13	65	F	605/13	BIOPSY-CERVIX	I	3	NEGATIVE	ABSENT	ABSENT
8	18242/13	69	F	606/13	BIOPSY-CERVIX	IB	2	NEGATIVE	ABSENT	ABSENT
9	18467/13	60	F	622/13	BIOPSY-CERVIX	IIB	2	NEGATIVE	ABSENT	PRESENT
10	18949/13	33	F	624/13	BIOPSY-CERVIX	IB	2	NEGATIVE	ABSENT	ABSENT
11	18725/13	55	F	651/13	BIOPSY-CERVIX	IIB	2	1+	ABSENT	PRESENT
12	19813/13	55	F	684/13	BIOPSY-CERVIX	IB	1	1+	ABSENT	ABSENT
13	24834/13	55	F	855/13	BIOPSY-CERVIX	IB	1	NEGATIVE	ABSENT	ABSENT
14	28012/13	61	F	968/13	BIOPSY-CERVIX	IB	1	1+	ABSENT	ABSENT
15	31494/13	60	F	1124/13	BIOPSY-CERVIX	IB	2	NEGATIVE	ABSENT	ABSENT
16	31437/13	60	F	1145/13	BIOPSY-CERVIX	IIB	2	NEGATIVE	ABSENT	PRESENT
17	33122/13	65	F	1146/13	BIOPSY-CERVIX	IIB	1	1+	ABSENT	PRESENT
18	33059/13	50	F	1149/13	BIOPSY-CERVIX	II B	2	NEGATIVE	ABSENT	PRESENT
19	33572/13	41	F	1187/13	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
20	35545/13	67	F	1241/13	BIOPSY-CERVIX	II B	4	NEGATIVE	ABSENT	PRESENT
21	35452/13	70	F	1242/13	HYSTERECTOMY	III A	2	3+	ABSENT	PRESENT
22	38234/13	60	F	1393/13	BIOPSY-CERVIX	I B	2	1+	ABSENT	ABSENT
23	47071/13	47	F	1622/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
24	45193/13	25	F	1743/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
25	44317/13	40	F	1744/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
26	46976/13	48	F	1782/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
27	44465/13	72	F	1805/13	BIOPSY-CERVIX	IB	5	NEGATIVE	ABSENT	ABSENT
28	48152/13	40	F	1831/13	BIOPSY-CERVIX	I B	5	1+	ABSENT	ABSENT
29	48268/13	40	F	1832/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
30	48439/13	45	F	1841/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
31	45196/13	66	F	1842/13	BIOPSY-CERVIX	IB	1	NEGATIVE	ABSENT	ABSENT
32	48440/13	30	F	1846/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT

33	47130/13	57	F	1848/13	BIOPSY-CERVIX	I B	1	NEGATIVE	ABSENT	ABSENT
34	50215/13	57	F	1932/13	BIOPSY-CERVIX	I B	1	1+	ABSENT	ABSENT
35	50973/13	43	F	1958/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
36	54130/13	67	F	2089/13	BIOPSY-CERVIX	I B	1	1+	ABSENT	ABSENT
37	54144/13	50	F	2090/13	BIOPSY-CERVIX	I B	2	1+	ABSENT	ABSENT
38	54515/13	60	F	2092/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
39	55508/13	63	F	2126/13	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
40	55387/13	53	F	2142/13	BIOPSY-CERVIX	I B	1	NEGATIVE	ABSENT	ABSENT
41	55881/13	62	F	2149/13	HYSTERECTOMY	III B	2	1+	PRESENT	PRESENT
42	56357/13	70	F	2167/13	BIOPSY-CERVIX	I B	1	2+	ABSENT	ABSENT
43	56896/13	63	F	2224/13	BIOPSY-CERVIX	II B	2	NEGATIVE	ABSENT	PRESENT
44	57624/13	53	F	2305/13	BIOPSY-CERVIX	II A	2	1+	ABSENT	ABSENT
45	57875/13	65	F	2356/13	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
46	59496/13	62	F	2365/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
47	59474/13	62	F	2366/13	BIOPSY-CERVIX	IIB	3	NEGATIVE	ABSENT	PRESENT
48	55073/13	74	F	2382/13	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
49	59235/13	39	F	2383/13	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
50	60635/13	50	F	2419/13	BIOPSY-CERVIX	I B	1	NEGATIVE	ABSENT	ABSENT
51	60334/13	65	F	2428/13	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
52	60983/13	67	F	2447/13	BIOPSY-CERVIX	II B	2	1+	ABSENT	PRESENT
53	61081/13	65	F	2449/13	HYSTERECTOMY	III B	2	2+	PRESENT	PRESENT
54	61076/13	40	F	2450/13	BIOPSY-CERVIX	II B	1	1+	ABSENT	PRESENT
55	58241/13	57	F	2518/13	HYSTERECTOMY	IIIB	3	3+	PRESENT	PRESENT
56	60575/13	65	F	2522/13	HYSTERECTOMY	III B	2	2+	PRESENT	PRESENT
57	62309/13	30	F	2523/13	BIOPSY-CERVIX	IB	5	NEGATIVE	ABSENT	ABSENT
58	62653/13	75	F	2545/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
59	58926/13	55	F	2555/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
60	62980/13	58	F	2560/13	BIOPSY-CERVIX	I B	1	NEGATIVE	ABSENT	ABSENT
61	67974/13	35	F	2623/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
62	18091/13	45	F	2624/13	BIOPSY-CERVIX	IB	5	NEGATIVE	ABSENT	ABSENT
63	65139/13	56	F	2644/13	BIOPSY-CERVIX	III A	2	1+	ABSENT	PRESENT
64	64724/13	56	F	2649/13	BIOPSY-CERVIX	I B	6	1+	ABSENT	ABSENT

65	64739/13	65	F	2650/13	BIOPSY-CERVIX	I B	2	1+	ABSENT	ABSENT
66	65427/13	53	F	2657/13	HYSTERECTOMY	III B	2	3+	PRESENT	PRESENT
67	64169/13	59	F	2666/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
68	65828/13	60	F	2673/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
69	65135/13	65	F	2711/13	BIOPSY-CERVIX	I B	2	1+	ABSENT	ABSENT
70	65962/13	70	F	2738/13	BIOPSY-CERVIX	II B	2	1+	ABSENT	ABSENT
71	65782/13	65	F	2771/13	BIOPSY-CERVIX	I B	5	NEGATIVE	ABSENT	ABSENT
72	68078/13	72	F	2776/13	BIOPSY-CERVIX	II B	2	1+	ABSENT	ABSENT
73	70607/13	47	F	2867/13	BIOPSY-CERVIX	I B	1	1+	ABSENT	ABSENT
74	72116/13	60	F	2912/13	BIOPSY-CERVIX	III A	3	NEGATIVE	ABSENT	PRESENT
75	71134/13	50	F	2914/13	BIOPSY-CERVIX	I B	2	1+	ABSENT	ABSENT
76	13079/14	62	F	640/14	BIOPSY-CERVIX	I B	2	1+	ABSENT	ABSENT
77	15038/14	50	F	761/14	BIOPSY-CERVIX	I B	2	2+	ABSENT	ABSENT
78	11779/14	70	F	832/14	BIOPSY-CERVIX	IB	1	1+	ABSENT	ABSENT
79	17790/14	75	F	881/14	BIOPSY-CERVIX	IIIA	5	NEGATIVE	ABSENT	PRESENT
80	17814/14	52	F	895/14	BIOPSY-CERVIX	I B	1	NEGATIVE	ABSENT	ABSENT
81	18578/14	58	F	917/14	BIOPSY-CERVIX	I B	2	1+	ABSENT	ABSENT
82	19141/14	50	F	975/14	BIOPSY-CERVIX	III B	1	NEGATIVE	PRESENT	PRESENT
83	19526/14	50	F	978/14	BIOPSY-CERVIX	III B	2	3+	PRESENT	PRESENT
84	22525/14	50	F	1136/14	BIOPSY-CERVIX	III A	2	2+	ABSENT	PRESENT
85	23336/14	40	F	1229/14	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
86	260124/14	52	F	1297/14	BIOPSY-CERVIX	I B	1	1+	ABSENT	ABSENT
87	25318/14	48	F	1300/14	BIOPSY-CERVIX	II B	2	NEGATIVE	ABSENT	PRESENT
88	26479/14	60	F	1395/14	BIOPSY-CERVIX	II B	4	2+	ABSENT	PRESENT
89	27080/14	80	F	1399/14	BIOPSY-CERVIX	II B	2	3+	ABSENT	PRESENT
90	26801/14	53	F	1419/14	BIOPSY-CERVIX	II B	2	1+	ABSENT	PRESENT
91	26899/14	60	F	1422/14	BIOPSY-CERVIX	II B	2	3+	ABSENT	PRESENT
92	27379/14	45	F	1428/14	BIOPSY-CERVIX	IB	1	2+	ABSENT	ABSENT
93	27059/14	53	F	1456/14	BIOPSY-CERVIX	I A	2	3+	ABSENT	ABSENT
94	28699/14	48	F	1458/14	BIOPSY-CERVIX	I B	2	NEGATIVE	ABSENT	ABSENT
95	28765/14	65	F	1462/14	BIOPSY-CERVIX	I B	4	NEGATIVE	ABSENT	ABSENT
96	28393/14	65	F	1517/14	BIOPSY-CERVIX	I A	2	NEGATIVE	ABSENT	ABSENT
97	29491/14	55	F	1527/14	BIOPSY-CERVIX	I B	1	NEGATIVE	ABSENT	ABSENT
98	26862/14	53	F	1554/14	BIOPSY-CERVIX	II A	2	3+	ABSENT	ABSENT
99	31327/14	67	F	1579/14	BIOPSY-CERVIX	I B	1	2+	ABSENT	ABSENT
100	32428/14	76	F	1643/14	BIOPSY-CERVIX	II A	1	NEGATIVE	ABSENT	ABSENT